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THE HARVEY SOCIETY

THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF
The HARVEY SOCIETY of NEW YORK
1926–1927

UNDER THE PATRONAGE OF THE NEW YORK
ACADEMY OF MEDICINE

BY

DR. FRED NEUFELD
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DR. LEONOR MICHAELIS
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SERIES XXII

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PREFACE

This volume of Harvey Lectures represents the twenty-second of the series. As in the past, the effort of the Harvey Society has been to obtain speakers whose work is distinguished and whose point of view is comprehensive.

The lecture by Professor Neufeld was previously published in the *American Review of Tuberculosis*. We are indebted to the Editor of this Journal for permission to reprint it.

CARL A. L. BINGER,
Secretary.

December, 1927.

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THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I

This Society shall be named the Harvey Society.

II

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV

The management of the Society shall be vested in an Executive Committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

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ORIGIN AND DISSEMINATION OF TUBERCULOSIS ACCORDING TO RECENT INVESTIGATIONS[1]

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IF,—WHAT is only too seldom done by the younger generation of bacteriologists,—one peruses Koch's paper published in 1884 on his discovery of the tubercle bacillus (1), one is surprised to find practically all the important problems engaging the attention of tuberculosis investigators of to-day, if not completely disposed of, at least there clearly set forth. Thus, the question of whether tuberculosis as a rule is transmitted by the bacilli entering by way of the lungs or by way of the pharynx and the nose; whether moist droplets or dry dust constitute the chief agent; whether the entrance of large numbers of bacilli has severer consequences than that of few bacilli; whether the germ of human tuberculosis is identical with that of bovine disease; and, at the same time, reference is made to the part played by predisposition, and to the particular difficulties encountered in combating tuberculosis, in consequence of its wide dissemination and the chronic course it takes. In reporting on some of the recent experimental observations on tuberculosis, I may, therefore, in almost all points link up with Koch's first investigations.

In regard to the question of how far, besides the lungs, the nose and pharynx can also be considered as portals of entry of the bacillus, Koch at the time expressed the view that tubercle bacilli might enter at these latter points only in the case of epithelial lesions. As is well known, numerous bacteriologists have up to the present defended the view that the normal mucous membranes and the normal skin are impermeable to microörganisms; nevertheless this assertion seems to have been refuted by numerous

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old and recent experiments. Koch, however, sets forth two special reasons in the case of tuberculosis: (1) that otherwise it could not be understood why so many individuals who come into frequent contact with phthisical patients could escape infection, and (2) the direct penetration of the tubercle bacilli by way of the lungs must be regarded as the normal route "because most cases of tuberculosis begin in the lungs." To-day we know, as a matter of fact, that in our countries most individuals in the course of their lives are infected with tuberculosis. As regards the second reason, it is well known how Behring, Calmette and others have emphatically maintained that human pulmonary tuberculosis is, as a rule, due to the penetration of the bacilli by way of the digestive tract. Even those who are not inclined to follow Behring's arguments will concede that phthisis is usually the final phase of an infection dating back years or even decades, and that, therefore, the question as to where the primary focus is to be sought, and how it originated, requires special investigation.

Great progress in this direction has been made by the discovery by Parrot and Küss of the "primary pulmonary complex" and its more intimate investigation by Albrecht, Ghon (2), and other pathologists. On the other hand, bacteriologists in Germany, especially Flügge (3) (4) and his pupils, and Calmette (5) and Chaussé (6) in France have endeavored, by means of exhaustive experimental research, to solve the question as to the pathways of infection, while in England the work of the Royal Commission on Tuberculosis, especially that of Stanley Griffith, has yielded important contributions on the subject. These researches have been completed by Bruno Lange (7) (8) in the Robert Koch Institute, and it appears to me that he has been successful in elucidating to a large extent the conflicting views of previous investigators.

In infecting guinea pigs and rabbits by inhalation, by feeding, by nasal and conjunctival instillation, and by rubbing the bacilli into the skin, Lange used, far more than his predecessors, exactly graduated doses down to the very limit of effectiveness; for only experiments with small quantities of bacilli may simulate a natural infection. At the same time, he made corresponding

experiments with a number of other bacteria (9), such as highly virulent streptococci and pneumococci, and the bacilli of anthrax, swine-erysipelas, chicken cholera, and mouse typhoid, which were also administered through the natural portals of entry. While these bacteria, when injected subcutaneously or intraperitoneally in even the smallest doses, acutely killed white mice, infection by inhalation, feeding, etc., gave very different results, and the outcome of these experiments elucidates the analogous but more complicated conditions in the case of tuberculosis.

Flügge was of opinion that, to infect animals by feeding, about one million times more tubercle bacilli were necessary than in the case of pulmonary infection, and in this he saw one of the main arguments why inhalation was the most important and most frequent route of infection. Lange has established that guinea pigs could be infected *per os* with very small doses, exceptionally even down to 0.000,001 mgm., which accounts for about 10 to 100 living bacilli. On the other hand, the subcutaneous, intraperitoneal and intratracheal administration of minimal quantities, the same which, when inoculated on a proper nutrient medium still gave rise to single colonies, regularly led to infection; and careful inhalation experiments revealed that also here apparently one single bacillus sufficed to produce an infection. Thus the digestive tract proved to be distinctly less susceptible. Far more important, however, are two further differences. The inhaling of even single virulent bacilli regularly brings about an infection, and in the case of the guinea pig this infection is in every instance an acutely fatal one. In both respects infection by way of all the other mucous membranes behaves differently: in so far as no excessive doses (such as practically seldom come into account) are given, only a certain part of the animals get infected, and further, the infection on an average, proceeds more slowly and is less severe, it shows a tendency to take a chronic course, and sometimes it may even heal spontaneously. It is, therefore, necessary to keep the guinea pigs many months under observation, to make frequent tuberculin tests, and to inoculate suspicious organs with doubtful lesions into healthy animals.

This fundamental difference may be explained in the following

way (10): If inhaled into the lungs the tubercle bacillus enters directly into a highly susceptible tissue, in which it retains all its original virulence and in which it rapidly develops. Therefore, in every case a typical focus is formed in the lungs, as well as in the corresponding lymph nodes ("primary complex"). In guinea pigs the infection progresses rapidly, and almost the same thing happens in a number of cases when quite young children are infected, while older children and adults even here show a high degree of natural resistance and, under the stimulus of the fully virulent and rapidly proliferating bacilli, produce powerful specific immunizing forces. Then the process in the latter comes to a standstill for the time being, and the organism retains permanently so high a degree of immunity that, when tubercle bacilli are afterward again inhaled they no more encounter a highly susceptible tissue, and so no other primary complex is developed. If the bacilli enter the body through one of the other mucous membranes or through the skin, they come here into tissues possessing high natural resisting forces from the beginning. In these cases, therefore, very often no primary focus develops at the point where the bacilli gained admission, by far the greater number of the bacilli being destroyed either in the mucous membrane or in the corresponding lymph channels. This, in my opinion, is the chief reason why such poor results are obtained after feeding with small or medium doses and such irregular results even with the larger doses. Those bacilli, however, which escape destruction and multiply in the lymph nodes, are to a large extent weakened in their virulence. Such weakened tubercle bacilli have been found in human beings in cases of lupus and verrucous tuberculosis of the skin, and in the lymph nodes (11), and sometimes also after experimental infection. May I mention an observation of Lange (12)? He found in a guinea pig, which had been infected three months before by way of the conjunctiva, suspicious swellings of the cervical lymph nodes and the spleen. The inoculation of a healthy guinea pig with the lymph nodes remained without result; inoculation with the spleen produced, after some time, several small nodules, which microscopically contained many tubercle bacilli, but which, inoculated into another guinea pig,

no longer caused disease. Perhaps such weakened bacilli are rather common and would be found often if it were not so difficult to cultivate them.

Analogous observations have frequently been made in the case of mice fed or infected by way of the nose or conjunctiva with septicemic bacteria, as indicated before. However, in the case of some bacteria, for instance pneumococci, the animals showed great resistance even against inhalation, a proof that the high susceptibility of the lungs in the case of tubercle bacilli is due not to mechanical causes but to biological processes. While a subcutaneous injection with the septicemic bacteria always caused acute death, after infection by the natural portal of entry the disease often took a slow course, extending over weeks and months, with remissions, and then it proved difficult to recover the bacteria from the organs, or occasionally one succeeded in cultivating modified virulent strains from the lymph nodes or the spleen. Animals with such chronic or latent infection,—for instance, with mouse typhoid bacilli or streptococci,—often die acutely when injected parenterally with small quantities of the same bacteria, so that it is evident that they possess no high degree of immunity. I believe we may assume that the same thing holds true in the case of tuberculosis, but, of course, I by no means wish to maintain that tuberculosis conveyed through food always takes a mild course. This is not so either in the case of the guinea pig or the human being as is shown by many fatal cases among children infected with bovine tuberculosis. I only wish to say that I believe that an infection by food, *on an average*, takes a much milder course and, *on an average*, is followed by a much weaker and shorter immunization than is an infection by way of the lungs. Indeed, in experiment, a treatment with nonvirulent bacteria frequently gives a very low immunity or no immunity at all; this has been proved not only in the case of streptococci and pneumococci but also in the case of tubercle bacilli.

This view is corroborated by the observations of pathologists. Beitzke (13) quite recently came to the conclusion that tuberculous infections by way of the digestive tract in many cases do not go further than to the "lymphoid stage," but are checked in

the beginning and, therefore, do not lead to any immunization. Beitzke further asserts that every *manifest* affection of a cervical or a mesenteric lymph node has just the same immunizing effect as a primary pulmonary complex. In this, however, I am unable to follow him, for the reason that in *all* infections there are all gradations of immunity present, ranging from complete absence of a specific protection to the highest degree of immunity attainable in the disease in question, and therefore the same thing is to be assumed in the case of tuberculosis. I therefore believe that also *manifest* lymph-node affections (in contrast to a primary pulmonary focus) are not always followed by a sufficiently high degree of immunity to prevent the originating of a typical primary complex after the inhalation of tubercle bacilli. Pathologists, as is well known, generally find only one such focus in the lungs; and they find it very frequently, some authors, indeed, in over 90 per cent of all tuberculosis patients. In view of the frequency of mild lymph-node tuberculosis among children, it seems improbable that such infection should, in none of these instances, already have existed when tubercle bacilli were first inhaled. So far as I may form an opinion, I further believe that manifest lymph-node foci frequently heal so completely that no traces of them are visible at autopsy.

Direct tuberculous infection by way of the lungs through inhalation accordingly occupies a special position, not because, as it was formerly believed, it is the most frequent, but because it is the severest form of infection. The question as to how frequently infections occur through the lungs as compared with the other portals of entry is difficult to answer; but in all probability infections by the latter paths are very numerous in children, and may sometimes be produced, as in guinea pigs, by but a few bacilli.

Now the bacilli of bovine tuberculosis practically never enter through the lungs, but nearly always through the other mucous membranes. It is also thought by many that they may enter through the skin and so cause scrofula of the lymph nodes. They have also often been found in cases of lupus, of tuberculosis of the bones and the joints, of fatal meningitis and miliary tuberculosis,

but only exceptionally in pulmonary phthisis,—altogether in 4 of about 1,000 investigated cases (14). Of these, one was a peasant girl and another a young butcher, people who probably came a good deal in contact with cattle, and who possibly could have *inhaled* bovine bacilli. From the fact that bovine infection is so rare in the case of pulmonary tuberculosis, may we not perhaps argue in favor of the assumption that this most frequent and important form of tuberculosis (the only one that practically at all comes into account in the spread of the disease from man to man) always takes its origin from a primary pulmonary infection and not from a focus in any other organ? I do not believe that we are yet in a position to give a final answer to this exceedingly important question. I only wish to point it out.

Lange (15) has quite rightly drawn attention to the fact that it is necessary again to raise the question concerning the virulence of bovine tubercle bacilli for man: if, *on the average*, they cause milder forms, is this not simply explained by the fact that they practically always enter through the pharynx and the intestines? Are human beings perhaps just as susceptible to bovine as to human tuberculosis? Against this one might point to the fact that bovine infection is prevalent only in children, and especially in young children, who, as we know, are, on the whole, less resistant to tuberculosis. This argument, however, is by no means convincing, because it is especially children who drink a good deal of unboiled milk; further, we do not know to what extent adults are susceptible to an infection through the digestive tract, even with human bacilli. If we were to adopt the view that the phthisis of the adult, in so far as it arises from infection in childhood, always proceeds from an old primary pulmonary focus, the almost complete absence of bovine tuberculosis in the case of phthisis could be accounted for, even without assuming any difference in virulence.

However this may be, we have every reason to guard our children against the dangers of bovine tuberculosis by suitable hygienic measures, above all by the pasteurization of milk. Only in the last decade have the important observations of Griffith (16), Hobday (17), and Fraser (18) shown how extensively bovine

tuberculosis is able to spread in those places where such measures are neglected. These observations are all the more important, as it is the bovine type of tuberculosis that can be prevented most easily by methods of public hygiene. On the other hand, the fact remains that so far as the most frequent and most important forms of human tuberculosis are concerned, bovine infection plays practically no rôle.

In our Institute a good number of lupus cases have been investigated since 1919, and the observations have been reported by Kirchner (11); and other more recent ones, not yet published, have shown about 50 per cent to be of bovine origin, a fact which, in my opinion, suggests the view that lupus, in most cases, may be a metastasis of an internal tuberculous focus, in contrast to *tuberculosis verrucosa cutis*, which shows quite different symptoms, and apparently takes its origin from an injured skin, thus corresponding to the percutaneous infections of animal experiment.

As is well known, Koch (19) in 1901, in establishing the view, held before him by Theobald Smith, that the infective agents of bovine and human tuberculosis are different from each other, not only made a systematic investigation of the occurrence of bovine infection in human beings, but also added that he considered such infections to be so rare that no special measures against them might be necessary. Because of the quite justifiable opposition which this statement in his London lecture aroused, we should not forget how important it was that a clear differentiation between bovine and human tuberculosis should just at that time have been established and the means indicated whereby both types of bacilli could be distinguished with certainty by animal experiment. A year later Behring set forth his theory that all phthisis of adults was due to an infection contracted in childhood, and to a large extent to infections acquired through milk; then followed the investigations of Roemer, Calmette and others. If, at that time, we had had no means of distinguishing bovine and human bacilli, we might indeed have believed that phthisis in our countries depended mainly upon bovine infection.

As we know, it is not possible to discriminate between the two types by means of agglutination tests. However, Schilling and

Hackenthal (20) have recently been successful in determining specific distinctions between human and bovine tubercle bacilli by the hypersensitiveness tests of Schulz and Dale. In their experiments the intestine of a guinea pig infected with a bovine strain gave a reaction, if not exclusively, at least in quite a preponderating number of cases, with a diluted extract of bovine bacilli only, and *vice versa*.

That children are much more susceptible than adults to every form of tuberculous infection, and the more so the younger they are, has been so conclusively proved by practical experience, that it requires no experimental corroboration. I will, therefore, only mention that the same difference has been observed upon feeding young and adult animals with several other species of bacteria, for instance, trypanosoma brucei (21), mouse typhoid, amebic dysentery (22). Tuberculosis in this respect is, therefore, not unique. So generally as this fact is known, it is far from being taken sufficiently into account in the practical measures against tuberculosis. Roemer (23) has claimed that our efforts in combating tuberculosis should chiefly be concentrated on the protection of children, especially during the first years of life, in order that the infection, which in many cases is scarcely avoidable, may be postponed as long as possible. This requirement must to-day still be regarded as entirely justified and should be placed in the foreground in all works of popular instruction. To be sure, this is one of the most important points of all measures of prophylaxis.

A second important claim, set forth by Roemer, has also been justified by recent observations. Roemer asserted (what Koch had already declared as probable in his first paper) that the final issue of a tuberculous infection depends, in large measure, upon the quantity of bacilli entering the body, and that it is generally the heavy and continuously repeated infections which sooner or later lead to a fatal end. In looking after families affected with tuberculosis we very frequently find that in seemingly healthy children the Pirquet reaction is positive; thus they are already infected, and we are then confronted with the question of whether it would still be of any use to guard them against further infection.

I believe we must answer this question in the affirmative. Köffler (24), Bräuning (25), and others followed over several years the fate of children who grew up in tuberculous families, and found in the surroundings of patients, whose sputum contained only a few bacilli or who followed the directions given them as regards cleanliness and preventive measures, far fewer cases of manifest disease, and among these far fewer deaths occurred than among children of those parents who emitted a large number of bacilli and who, in addition, were careless and unclean. Redecker (26) has recently described pathological observations, according to which old tuberculous foci again became active after renewed infection. Such observations indicate clearly the danger of repeated infections. Roemer's further assumption that heavy infections in childhood are one of the chief causes of subsequent phthisis has a good deal of probability though it may be difficult to prove it directly. In animal experiment it is also found that if smaller doses of tubercle bacilli are added to the food, the infection (though not in every individual case, but on an average) takes a much milder course than if large quantities of bacilli are administered. In the case of other infectious agents, especially in the very numerous experiments made with mouse typhoid, the same law has been observed.

If, therefore, recent observations lead us, in the case of tuberculosis, to ascribe a great influence to the *quantity* of the infectious material, we practically come back to the requirement repeatedly advanced by Koch that, first of all, the advanced phthisical patient, who expectorates great quantities of bacilli, should as far as possible be segregated. This remains, as heretofore, the most important of all measures.

What a different course tuberculosis takes in surroundings, in which there is no opportunity whatever of receiving large quantities of tubercle bacilli, and particularly of inhaling them, is indicated by the remarkable observations of Hillenberg (27) and Jacob (28), which up to the present have not, I believe, been given sufficient attention. By means of tuberculin tests they found in remote villages of Germany, with little traffic, in which for many years no case of open tuberculosis had occurred, 25 to 40 per cent

of the children infected, but none of them manifestly ill. In whatever manner this infection may have been brought about, we may at any rate assume that only small quantities of bacilli were active in each case; perhaps, also, mainly or exclusively intestinal and not pulmonary infections were involved. In my opinion it would be highly desirable if these investigations were continued at other suitable places, and supplemented in such a way that the ages of first Pirquet reactions be noted and that in cases, in which intercurrent deaths occurred, an exact pathological examination be made in each instance. Should a tuberculous focus be found, it should be inoculated into animals, to determine the type and virulence of the bacilli.

If we ascribe a special significance to the direct infection of the lungs through inhalation, in contrast to all other forms of infection, the old question, of whether dry dust particles or moist droplets play the chief part, gains renewed interest. On this point Koch, in his first paper on tuberculosis, expressed the view that infection by particles of dust was the usual form in which the disease is conveyed, "the sputum particles being as a rule not so small that they could remain suspended in the air for any length of time." This view was entirely thrown into the background, at least in Germany, by the numerous experiments of Flügge and his coworkers (3) (4), who saw in the droplets coughed out by phthisical persons and immediately inhaled by healthy individuals, the chief agent of infection. Flügge's conclusions, however, have been refuted by the careful research work carried out by Lange (29) in the last few years, which I have just reported in a lecture at the National Tuberculosis Association meeting in Washington.[2] These investigations tend to prove that the danger of coughed-out droplets has been overestimated by Flügge, and, on the other hand, sputum particles as well as droplets deposited on handkerchiefs, clothing, or on a dusty floor, are often within a few minutes dried up, so that they can easily be shaken off and remain suspended in the air. In this state they are easily inhaled by the

[2] The significance of cough droplets and dry dust in the spread of tuberculosis, *American Review of Tuberculosis*, 1927, xv, 609.

experimental animals, and then cause the typical primary pulmonary foci.

This widens to a considerable extent the scope of our prophylactic measures and renders them more difficult. We must attempt to safeguard the neighborhood of a coughing patient, not only against contamination from his direct coughing, but also against what is perhaps the greater danger, the droplets and sputum particles scattered on the clothing, linen, bed, and sometimes also the furniture and floor.

If, as the chief results of recent investigations, I have indicated the special importance of pulmonary in comparison to other modes of infection and, on the other hand, the danger of quantitatively heavy infections, I must once more emphasize the fact that I only desire to state that, on an average, the one mode of infection has graver consequences than the other one. The progress of the disease in each individual case depends, to a large extent, upon other circumstances, above all upon the constitution, that is, the natural protective forces of each individual and his capacity for elaborating specific protective substances. In both respects, individuals, excepting perhaps young children, show such great differences that these constitutional factors are perhaps to be regarded as the most important of all. This fact was already known to the ancient physicians, and the more recent experiments referred to above, in which animals were infected through the natural ways with small doses, corroborate it; we then see the same manifold forms of disease as in the natural course of an epidemic.

As the most important factor after the innate constitution must be regarded the external circumstances which influence constitutional disposition, above all food; and here it seems not alone to be a matter of quantity, but also whether certain substances are present or lacking. Whether vitamines may here play a rôle is not yet ascertained. In any case, some experiments on guinea pigs, especially those of Bieling (30) and Heymann (31), have shown that a scurvy-producing diet exercises an unfavorable influence on tuberculosis. From further experiments in this direction, important practical results may perhaps be expected.

In this connection I would draw attention to the remarkable

observations made by Planner-Wildinghof (32), who described a singular form of tuberculosis seen in several hundred German soldiers, who were prisoners of war in a Siberian camp where very unfavorable conditions prevailed. The course of the disease was acute, and in most of the cases fatal. It proceeded in the form of a lymph-node tuberculosis, in some instances with caseation and enormous swellings, miliary patches, frequently also with intestinal tuberculosis, but no phthisis of the lungs. Probably we may have to seek in some particular defects of food the reason which caused the resisting powers in these individuals to fail to such an extent that they behaved like men of primitive tribes untouched by tuberculosis or quite young children in our countries.

If new investigations have demonstrated that the epidemiology of tuberculosis follows the same general laws as other infectious diseases, they have, on the other hand, corroborated the fact that in combating tuberculosis we must take account of the peculiarities the disease offers as an eminently chronic and, at the same time, almost universally prevalent infection. From the point of view of the bacteriologist, we emphasize the special significance of direct pulmonary infection; the knowledge that dry dust here plays an important rôle, the special dangers of large and repeated doses of infections, and the exceptional susceptibility of early childhood. The chief influence on the course of the disease must, however, be ascribed to the constitutional factor, and to the influence exerted on this factor by food, housing and the whole mode of living. Thus, in combating this disease we shall find confirmed anew the words with which Koch concluded his paper on *The Etiology of Tuberculosis,* namely that in all measures undertaken against tuberculosis we must always take into account the social conditions.

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THE NATURE OF THE LIVING CELL AS REVEALED BY MICRODISSECTION[1]

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THE science of cytology owes its birth to investigators who were primarily experimentalists. When the names of Dutrochet, Dujardin, Brown, Schleiden, Von Mohl, Berthold and Pfeffer are called to mind we immediately visualize men who concerned themselves not so much with observations as with methods of experimentation.

The investigations on the cell were seriously hampered, however, by its minuteness and by the apparent homogeneity of its protoplasm. When, therefore, artificial methods were discovered which brought into evidence, in the killed cell, structures of ever increasing complexity, the subject was more and more taken over by the morphologist. Even today the term "cytology" expresses little more than a structural study of killed and stained cells. The pendulum is now swinging back to the experimental method and there is accumulating a wealth of literature which deals with experimental cellular biology and cellular physiology.

Extended studies have been made on the effect of subjecting living cells to different environmental conditions, and our knowledge of the permeability of protoplasm and the action of electrolytes, especially those found in physiological fluids, has been considerably advanced. Most of the results obtained in these studies are based on the indirect method of dealing with masses of cells. The more direct method is to deal with the individual cell.

Owing to the peculiar nature of protoplasm it can be studied only within microscopic dimensions and the most direct method

[1] Lecture delivered November 20, 1926.

of doing this is to use contrivances which permit manipulation within such confines.

Attempts have long been made to operate on the cell by mechanical means. Probably the first person to tear the nucleus out of a cell was a southern physician, Dr. Henry Schmidt, who lived in New Orleans before the Civil War. This investigator used steel needles, scalpels and fine scissors mounted on contrivances equipped with universal joints. In a pan of weak alcohol he dissected out the bile capillaries of the human liver and even tore up the liver cell and isolated its nucleus. His first paper on this subject was published in the *American Journal of the Medical Sciences* in 1859. A reprint of another paper was published in the fourth volume of the *Transactions of the Royal Microscopical Society* in 1870.

It was many years later that we again hear of attempts at microoperative technique on cells when Chabry, a Frenchman, in 1887, made minute glass needles which he shot with a hair trigger mechanism into living egg cells.

However, it was not until comparatively recently, between 1904 and 1910, that McClendon, in New York, Barber, in Kansas, and Schouten, in Holland, independently of one another, elaborated a technique which enabled one to manipulate with exceedingly fine glass needles and pipettes in the field of the higher magnifications of the compound microscope.

Barber and Schouten used their technique almost exclusively for isolating microörganisms. This was done with micropipettes which were made by carefully breaking off the tips of hollow glass needles. In 1912 Kite used needles with unbroken tips and was the first to apply the technique to a study of the physical properties of protoplasm. Since then the method has been further developed with improvements in the construction of the needles and pipettes and of the instruments for manipulating them.

There are now three types of micromanipulators which are in most common use (1, 2, 3). Recently developed accessories of greatest importance are microelectrodes (4, 5) and mechanical devices for microinjection. With such instruments at hand there seems to be no end to the opportunities for experimenting with

protoplasm by the direct manipulative method. It lends itself not only to investigations in cellular physiology but to problems in pure physical chemistry, e.g., the physical state and viscosity of emulsions (6) and colloids (7, 8). The method is also being used to advantage in the study of the physiology of urinary secretion (9, 10) and of the function of blood capillaries (11, 12).

The results from the micromanipulative technique on more purely cellular phenomena may be listed under the following headings.

I. EXPERIMENTAL EMBRYOLOGY

McClendon (15) and Kite (13) were pioneers in the microdissection of living cells. During the few years before his death in 1918 Kite did a great amount of work most of the results of which were never published. In a lecture delivered at Woods Hole in 1914 he described graphically an extraordinary operation which he performed on the sperm and egg nuclei of a tropical sea urchin egg whose diameter is less than 0.1 mm. These results I have since verified on the almost equally translucent sanddollar egg. The sperm and egg nuclei are minute spherical bodies considerably less than 0.005 mm. in diameter. Upon insemination of the egg there occurs a march of events not the least remarkable of which is the migration of these nuclei toward each other and their ultimate fusion to form the nucleus of the segmenting egg. Kite attempted to prevent this fusion both by interposing the tip of a microneedle between them and by seizing one nucleus and dragging it away. In each case the nucleus, when released, resumed its normal course. This experiment of Kite's is an illustration of the minuteness of the operations which are made possible by this technique.

One of the most puzzling phenomena in the segmentation of the egg has been the formation of astral configurations which have been likened to magnetic fields of force controlling cell division. By means of microdissection it has been demonstrated that these asters are gelated regions of the protoplasm (14). It has been possible to move them about within the cytoplasm of the living egg, and, by shifting them to new positions, to change the original

plane of cleavage. Drastic mechanical agitation causes them to revert to a fluid state and to disappear, whereupon cell division is prevented.

Among other studies may also be cited the results of removing the nucleus from the egg (15, 16) and the effects of such removal on hybridization (17).

II. EXPERIMENTAL PROTOZOOLOGY

Experiments on ciliates have demonstrated that the micronucleus is essential for life and has given proof for the neuromotor function of differentiated fibrils in Euplotes (18, 19). Extended studies have also been done on the behavior and function of the contractile vacuole (20, 21).

III. EXPERIMENTAL HISTOLOGY OF SURVIVING TISSUES

The physical state and relationships of various metazoan cells have been investigated and the existence of definite intercellular bridges between the cells of the human epidermis has been definitely established (22). Cutting and tearing experiments also have been made on the processes of nerve cells in tissue culture (23).

The structure and mode of action of cilia and cirri (24, 25) and of ciliated epithelium have also been investigated. Recently the writer has been studying the physical state of the epithelium in the glomeruli of the normal and diseased human kidney.

Intracellular structures, viz., the nuclear spindle, the mitochondria and chromosomes have been microdissected (26, 27, 28). The mitochondria at certain stages are exceptionally resistant fibrils which can be dragged out of the cell. The spindle is a formed body which can be pushed about within the cell except when it is anchored by asters which develop at its poles (14). No spindle fibers can be demonstrated as concrete structures and the chromosomes appear to be free within the matrix of the spindle. The chromosomes are jelly-like structures which can be stretched and torn.

IV. MICROCHEMISTRY IN MICROSCOPIC DIMENSIONS

This field is being developed by H. Pollack in this laboratory. Chemical reagents in microdroplets of approximately five cubic microns in volume can be handled easily in the field of the microscope under a magnification of 1000 diameters. Mr. Pollack is applying this method to intracellular chemistry.

V. PHYSIOLOGY OF PROTOPLASM

A study of protoplasm is one to which the micrurgical technique particularly lends itself. I wish, therefore, to dwell at length on some of the main results accruing from work in this field to which a group of us are devoting ourselves at Cornell University Medical College and the Eli Lilly Research Division in the Marine Biological Laboratory at Woods Hole.

The evidence is strong that the material composing the surface of protoplasm differs markedly from that of its interior. These two essential parts of protoplasm, therefore, are considered separately.

a. The surface of the cell and of protoplasm

Not only plant cells but many animal cells possess extraneous walls or pellicles (29) which hold within their confines the protoplasm with its own true plasma membrane or plasmalemma. In plant cells the cellulose walls are evident and usually quite rigid. The cellulose wall is covered with a layer of pectin-like substance which forms the adhesive material binding the cells together. With suitable reagents this layer can be softened or dissolved so that the cells tend to fall apart.

In animal cells the cellulose constituent of the wall is absent, but a layer analogous to the pectin-like constituent exists and serves as an intercellular cement. This cement is developed to an appreciable extent between the blastomeres of the marine sea urchin eggs. In normal sea water the adhesiveness of this cement renders the separation of the contiguous blastomeres almost impossible. However, they can be separated easily by immersing the eggs in sea water which lacks the bivalent electrolytes, calcium

and magnesium. The cement then softens and tends to dissolve, whereupon the cells fall apart. If the cells are returned to a medium containing calcium, the substance, which the protoplasm is continually exuding over its surface, stiffens and furnishes them anew with a coating of adhesive, binding material.

In free living cells this extraneous coat may or may not be appreciable. In many protozoa it is sometimes stiff enough to form a pellicle which can be stripped off by means of microneedles (29).

The protoplasm within the cell possesses its own bounding surface. This is readily seen in plant cells where there is a sharp line of demarcation between the protoplasm and the large central vacuole. It is also evident when the protoplasm has been made to plasmolyze so that it shrinks away from its cellulose wall.

The most serviceable material for a microdissection study of the physical properties of the naked surface of protoplasm, is to be had from mature, unfertilized, Echinoderm eggs. An egg is seized with one microneedle and its surface torn with another. As the protoplasm exudes through the tear it quickly forms a film-like membrane which separates it from the surrounding sea water. Even this in time becomes coated with an appreciable layer of jelly. For the first few minutes, however, it may be considered as a truly naked, protoplasmic surface membrane. That this layer is a true membrane and not merely an interface between two immiscible fluids can be demonstrated, by tearing it rapidly with a needle. A wave of disintegration then spreads over the surface which destroys it so that all appearance of a sharp demarcating line is lost. The interior then scatters and dissipates in the surrounding medium (30).

Calcium is essential for the formation and maintenance of the protoplasmic membrane. If an egg is torn in a solution of pure sodium chloride which is isotonic with sea water, the break in the surface is not repaired and the granular contents simply pour out and become dissipated. On the other hand, an egg, in an isotonic solution of calcium chloride, develops a marked capacity for maintaining an intact membrane. When immersed in such a solution the eggs can be rolled about and pinched into droplets

each of which is covered with its own membrane. These membranes are fluid and the spherical fragments of the eggs roll about much like oil droplets. Even in the case of the ameba, which ordinarily lives in fresh water, calcium chloride has been found to increase the reparability of its surface membrane while sodium chloride inhibits and disperses it (30).

The highly fluid state of the naked protoplasmic membrane can be demonstrated from the following experiment on the sea urchin or starfish egg. With a delicate microneedle the naked surface of a freshly formed exovate can be seized and dragged out for some distance in the form of a filament with the needle holding the tip. On reversing the movement of the needle so that it approaches the surface of the exovate the substance of the filament flows back into the exovate with no sign of wrinkling on its surface. If the filament is stretched beyond an optimum it may break into a string of coherent droplets. On releasing the tension the droplets at once flow together and the filament reverts to its original cylindrical shape.

A striking experiment which offers a suggestion regarding the nature of the plasma membrane is given in the following experiment on an actively flowing ameba, the plasma membrane of which is almost naked. On being dragged out of the water, the plasma membrane will be caught by the surface of the water and will be pulled entirely off the ameba (30). The ameba, which has in this way literally been flayed alive, may recover if it is immediately pushed away from the surface into the depth of the water. Surface films then quickly begin to form in the outer zone of the protoplasm which is in imminent danger of becoming dispersed. The recovery of the ameba depends upon the ability of the surface films which are shooting back and forth to unite and form an intact investing membrane. During this procedure a considerable amount of the substance of the ameba escapes and is dissipated.

b. The interior of protoplasm

The difference in the appearance of the protoplasm of different types of cells is due largely to variations in the character of the

granules and vacuoles suspended in an optically structureless matrix. When the vacuoles are numerous and crowded together the protoplasm is frothy and tends to be firm in consistency. The consistency of protoplasm may also vary through changes in the hyaline matrix which are analogous to those occurring in the formation of sols and gels in colloids. The ultramicroscopic appearance of this matrix and the reversibility of its physical state are some of the properties by virtue of which it is classified as a colloid.

Evidence from experiments on the injection of aqueous and non-aqueous solutions into such diversified cells as the ameba, the pancreas cell of the frog, and marine ova, clearly indicates that their internal protoplasm is freely miscible with aqueous solutions. In small quantities water diffuses readily through the protoplasm without affecting its viable state. In larger quantities it induces destructive changes (30).

The injection of solutions of the chlorides of sodium, potassium, calcium and magnesium has thrown considerable light on the importance of electrolytes in protoplasmic functions. The salts of the monovalent electrolytes, sodium and potassium increase the dispersion of the internal protoplasm. This is similar to their effect on the plasma membrane. On the other hand, the injection of solutions of $CaCl_2$ or of $MgCl_2$ produces a coagulation. This is in striking contrast to their effect on the plasma membrane upon which they have no coagulating action (30).

These phenomena emphasize two facts of great significance concerning the action of electrolytes on protoplasm. One fact is the profound difference between the plasma membrane and the internal protoplasm. The other fact offers an explanation for the mutual antagonism of mono- and bivalent cations both on the internal protoplasm and on the plasma membrane, viz., the normal physical state of the internal protoplasm can be maintained when the bivalent and monovalent cations are present in certain proportions because the solidifying action of the one will balance the dispersing action of the other. Similarly the antagonistic action of the electrolytes on the plasma membrane is due to the balance between the stabilizing action of the bivalent and the dispersing action of the monovalent cations.

This fact is in harmony with the interpretation of protoplasmic systems given by Clowes in his experiments on the action of salts on water and oil emulsions (31). The calcium salts favor the formation of an emulsion of water in oil and would therefore enhance the maintenance of a lipoid-like film on the surface of protoplasm. The toxic action of the sodium salts on such a film is due to their dispersive action on the lipoid by breaking it up into an emulsion of oil in water.

Upon the intact condition of the plasma membrane depends the integrity of the interior of protoplasm and the very salts which are least injurious to the surface and upon which the surface depends most for its maintenance are the salts which are most injurious to the interior.

In the absence of salts the protoplasm of Echinoderm eggs coagulates. This can be shown by plunging the eggs with a minimum of sea water into a large quantity of distilled water. The eggs begin to swell by osmosis but before the surface breaks the entire mass of the egg sets into an irreversible coagulum.

We may state, therefore, that the salts maintain the peculiar colloidal state characteristic of living protoplasm, and the relative amounts of the two types of salts present are so proportioned that the associative or solidifying action of one is offset by the dispersive action of the other.

c. The pH of protoplasm

The importance of acid-base conditions on the action of electrolytes and on protoplasmic activities in general led to a study of the hydrogen ion concentration of the cell interior. A considerable amount of work has been done on this problem without the aid of the micromanipulation technique. Of the published data, however, comparatively few can be considered to deal strictly with the intraprotoplasmic pH. The results of even these few are open to criticism because of the possibilities of error from injury to the tissues in the procedures employed.

A case in point is the use of fluid extracts from crushed tissues. Not only is there a possibility of error from crushing cellular tissues but also from mixing of intracellular extracts with the

highly buffered extracellular fluids which can never be completely eliminated from a mass of tissue. There are also serious objections in attempts to determine pH by exposing cells to solutions of indicator dyes either by immersion or by injecting the dyes into the circulation of an animal. One of the objections is the impermeability of normal living cells to the vast majority of useful indicator dyes. The other is the fact that those dyes which do penetrate, e.g., neutral red, almost always become adsorbed on or accumulate within intracellular inclusions, such as granules and vacuoles, and give variable indications irrespective of the true pH of the protoplasmic substratum.

By means of the micromanipulative technique both potentiometric and colorimetric methods are conceivably possible for determining the pH of protoplasm. The electrometric method is still in the experimental stage.[2]

Definite results have been obtained with the colorimetric method by injecting solutions of indicator dyes into the protoplasm. The series of sulphonephthalein indicators (37) and the basic dyes, neutral red and methyl red, have proved to be most serviceable for this purpose. They are prepared in aqueous solutions of sodium salts, and, when injected into the protoplasm, readily permeate it. This is in accordance with results already obtained with other sodium salts (30) which maintain the fluid state of the protoplasm and allow the ready diffusion of the injected solution throughout the cytoplasm. Fortunately the dyes are relatively non-toxic. This is specially true for phenol red, the useful pH range of which happens to include the pH value found for protoplasm. Amebae, for example, after the injection, maintain the color of the dye and live in a normal condition for days. An important consideration regarding the validity of the colorimetric method for determining the intraprotoplasmic pH is the fact that all the solutions of dyes, both acid and basic, have been found to give consistent indications, when injected, irrespective of whether the dissociation of the dyes is at its lowest in their acid or alkaline ranges (32).

[2] Taylor and Whitaker have very recently reported results on the potential difference between the cell interior and outside medium (40).

Colorimetric determinations by means of microinjection have already been reported by several investigators. Schmidtmann (33) introduced solid granules of dyes into mammalian cells where the granules went into solution and gave characteristic color reactions. Her findings of the pH in the cells of different tissues give values varying from 6.3 to 7.5. It is too early to decide to what extent these variations are due to the presence of secretory products in the cells rather than to inherent differences in the true protoplasmic pH.

The Needhams (34) injected solutions of dyes of the Clark and Lubs' series into *Amoeba proteus* and recorded its pH to be between 7.4 and 7.6. From experiments conducted in this laboratory we place the pH of the ameba at 6.9 ± 0.1. In our experiments the injected amebae survived the injection and maintained the characteristic color for days.

The most unequivocal results obtained so far are those on the starfish egg in which our findings (32) agree with those of the Needhams (35) in placing the cytoplasmic pH at a figure within one or two decimal points of 6.8.

We have also determined the pH of the living nucleus to lie between 7.6 and 7.8. This determination was made on the germinal vesicle of the living starfish egg, which, after the injection, underwent normal changes preparatory to segmentation.

Recently we have used the same method on cells from the body of the frog and Necturus, e.g., active and resting muscle, epithelial and glandular cells (36). The injection of these cells indicates a fairly constant pH value of 6.8 to 7.0 for the cytoplasm, and 7.6 to 7.8 for the nucleus.

All these experiments point to the probability that the cytoplasm of living cells in general has a reaction which is constant within fairly narrow limits. The nucleus of different types of cells also shows a similar uniformity, viz., a constant hydrogen ion concentration of about 7.6 to 7.8. Another fact of interest is that the pH of the protoplasm of cells is distinctly more acid than their surrounding media. For example, the marine ova with an intracytoplasmic pH of about 6.8 live in sea water the pH of which is about 8.4. Metazoan tissue cells are bathed in

tissue fluids and blood which have a pH of about 7.5 and fresh water amebae thrive best in water having a slightly alkaline reaction.

d. The oxidation-reduction potential of protoplasm

The micromanipulative method has also made possible an investigation of another most important phase of cellular activity, viz., the oxidation-reduction potential of protoplasm. A determination of this factor will probably prove to be of great value in a study of the respiration and metabolism of the cell.

Clark, Cohen and co-workers (37) have recently elaborated and standardized a number of indicator dyes which differ in the ease with which they can be oxidized and reduced. These dyes are arranged in a series in the order of their ease of reduction (in terms of the electrode potential of their equilibrium system). The dyes are mainly the indophenols and the indigotines. The indophenols constitute a majority of those dyes which are more easily reducible than methylene blue while the indigotines are reduced with greater difficulty. As a measure of reduction intensity Clark chose the symbol rH which is the logarithmic value of the hypothetical molecular hydrogen pressure in equilibrium with the oxidation-reduction system in question. Just as pH refers to the intensity of acidity in distinction to the total amount of acid present, so rH refers to the intensity of reduction in distinction to the total amount of reductant or oxidant present. The computed rH value has definite significance only when the pH is constant for any set of comparisons.

Therefore, in order to determine the rH or the oxidation-reduction intensity of a system one must know not only which of the dyes are completely, partially or not at all reduced but also the pH of the system at the time when the determination is being made.

To the Needhams of Cambridge, England (34, 35, 38), is due the credit of realizing the possibility of determining the intraprotoplasmic rH by means of microinjection. They selected, as material, fresh water amebae and the eggs of starfish and sea

urchins. After having determined the pH of the ameba to be about 7.4 and that of the Echinoderm egg to be 6.8, they prepared the oxidation-reduction dyes at a pH of 7.0 and determined the rH of the ameba to be 17–19 and that of the eggs to be 21–22. Thus they were able to demonstrate the existence of a definite degree of the intensity factor for oxidation-reduction in protoplasm. In other words the oxidation-reduction activity is poised at a fairly constant level quite analogous to the maintenance of the hydrogen ion concentration in a well buffered system. Moreover the intensity factor can be expressed in terms of quantitative values. Their results indicate that this intensity factor differs in different cells for it appears to be distinctly greater in the fresh water ameba than in the marine starfish egg. According to the Needhams the oxidation-reduction intensity in anaerobic protozoa is greater than that in aerobic forms, such as the ameba. However, they found that in the latter the intensity is maintained at the same poised level under both aerobic and anaerobic conditions. This is rather extraordinary when considered in the light of the observations of Cannan, Clark and Cohen (39) on cell suspensions in which anaerobic conditions pronouncedly change the rH.

The Needhams' results require careful study before a satisfactory interpretation can be placed upon them, and a determination of the oxidation-reduction potential within the protoplasm of a cell should be amply checked from various sources. Therefore, with the aid of Dr. Cohen a group in our laboratory is now engaged in an extended study along this line.

e. Cell division

There is still another field of cellular activity which is amenable to investigation by means of the micromanipulative technique. Very little is known concerning the factors which control cell division, an explanation of which should go far toward a solution of some of the most pressing problems in medicine. Before we can hope for much, however, we must first build up an adequate conception of the varied physiological processes of the cell, some of which have been reviewed in this lecture.

The existence of definitely directed currents of flow in the cytoplasm of the segmenting ovum has already been described by several investigators who have suggested that the formation of the cleavage furrow is due to vortical movements in the equatorial region of the egg (cf. 28). These currents are best seen in rapidly dividing cells such as the nematode egg. In the more slowly dividing sea urchin egg they are barely appreciable but can be strikingly demonstrated by taking a time exposure of an egg which has been properly oriented to the lens of a camera. The developed picture exhibits numerous curved streaks caused by the movement of the cytoplasmic granules along the paths of the currents. These currents flow over the surface of the egg from the two opposite poles. At the equator the two opposing currents turn inward producing a vortex which is concomitant with the sinking in of the equatorial furrow. This phenomenon resembles that of an oil drop which is made to divide by lowering the surface tension at two opposite poles, and has led some investigators to suggest that surface tension forces play an important rôle in cell division.

The furrow which forms at the equator of the oil drop divides the drop in two only when the two daughter droplets are free to move apart. If, on the other hand, the opposing surfaces of the deepening furrow become contiguous they merge, and the droplet resumes its original spherical shape. Such is not the case in the normally dividing cell for the furrow frequently advances with its sides in such close contact as to give the appearance of a thin line. This dissimilarity between the dividing oil drop and the cell has been used as an argument that a comparison between the two is unjustified.

It is of interest to note that the sea urchin egg can be placed under conditions in which this dissimilarity is largely eliminated. This is brought out in the following experiment in which eggs were allowed to segment in a solution of the salt of a monovalent cation, potassium chloride, isotonic with sea water. As the cleavage furrow deepens, the two daughter cells roll apart in a manner similar to the behavior of the two halves of a dividing oil drop. If the egg is held with needles so that the daughter halves

cannot roll apart, the sides of the furrow between them become contiguous and merge, and the egg remains undivided. The cytoplasmic currents continue unchecked, so that the furrow may repeatedly reappear and disappear.

The division of the oil drop is initiated by lowering the surface tension at the two poles of the drop with respect to its equator. This is done by the application of sodium carbonate simultaneously at the two poles. In the case of the egg cell normal division is always preceded by definite karyokinetic changes of the nucleus, accompanied by the formation of astral radiations in the cytoplasm. When once these changes have taken place, no mechanical disturbance, short of that which actually destroys the asters, will impede the division of the cell (28). However, if we exclude the factors which initiate the cytoplasmic currents and take into consideration only the part taken by the currents in the division of the cell the resemblance between the dividing oil drop and the egg in potassium chloride is strikingly close.

The variation between the behavior of eggs immersed in potassium chloride and those in their normal environment of sea water containing calcium may be accounted for as follows. The protoplasmic surface of the Echinoderm egg is coated with a thin layer of glutinous jelly, secreted by the egg. This is the pellicle which, as mentioned in the earlier part of this lecture, is more or less universally present on living cells. Any newly formed surface, e.g., the sides of the cleavage furrow, exudes a substance which, in the presence of its normal, calcium-containing environment, immediately sets to form a jelly. In the absence of the bivalent cations, calcium and magnesium, the material either dissolves or is softened and washed away. In a solution of potassium chloride, therefore, the egg can divide only when the opposite sides of the furrow do not come into contact. Otherwise, there is nothing to prevent their merging with the consequence that the furrow disappears. In normal sea water, on the other hand, the furrow fills with a jelly as fast as it sinks into the egg. The jelly separates the protoplasmic surfaces bordering the furrow, and prevents their mergence, thus enabling the cell to divide. This phenomenon lines up with our knowledge concerning the

need of calcium for the growth of tissues in general. We can now see that in the growth process, one of the requirements which is filled by calcium is to stiffen the intercellular substance. This increases the chances of cell division and also enables the cells to adhere so as to form a coherent tissue.

This lecture is a statement of the varied phases of cellular activity which are being dealt with by a new method of approach. Any adequate concept of the life processes requires a thorough knowledge of the action of the essential electrolytes not only on protoplasm as a whole, but on its various constituents. In addition to this, and intimately related to it, is the need for a comprehension of the acid-base equilibria and the oxidative processes in the protoplasmic system. All of these contribute to the maintenance of the structural and functional integrity of protoplasm. Through the micrurgical technique we have a direct method for attacking these problems. This technique offers an opportunity to develop such a conception of the dynamic living cell as has not heretofore been possible.

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SOME PROBLEMS CONCERNING THE GASTRIC JUICE[1]

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THE secretion of the mucosa of the stomach differs from any other liquid of the animal body by its very strongly acid reaction. All the other kinds of tissues cover a range of pH between about 6 and 8, just in the vicinity of the neutral reaction which corresponds to a pH of about 7. The most alkaline liquids such as the intestinal secretion never exceed the alkalinity of a solution of sodium bicarbonate; there is even no probability of a tissue fluid in which no free carbonic acid is present besides the bicarbonate. The most acid liquid of the body may be the juice of a striated muscle under anaerobiotic conditions when much lactic acid is accumulated, or the juice of a muscle taken out from a freshly killed animal in which, after the cessation of the blood circulation, the possible maximum of lactic acid will be developed. Here a pH of about 5 may be reached. It is only in the gastric juice that an acidity may be reached corresponding to a pH between 1 and 2, the average being at the culmination of the gastric digestion about 1.7, that means the same concentration of hydrogen ions as in a 0.02 normal solution of hydrochloric acid. In the gastric juice of the dog, a pH = 1.0, or the acidity of a 0.1 N HCl is easily reached.

The physiological significance of this great acidity is well-known. It is the pepsin which requires such a high acidity for its activity as a proteolytic enzyme.

Taking into consideration the gastric juice with its high acidity and the pepsin contained in the stomach during the period of digestion, the first problem of the gastric juice which has perplexed investigators for a long time is: Why does not the acid

[1] Lecture delivered January 22, 1927.

pepsin-containing gastric juice digest the wall of the living stomach itself? It seems to me that this problem no longer involves any difficulties. It is virtually certain, from a histological aspect, that the acid and the pepsin, though produced in the same glands, are not produced in the same cells. Some cells may secrete acid, others pepsin. The mixing takes place only in the cavity of the stomach. Now the superficial cell membranes, which regulate the permeability of any cell, seem to consist of some lipoid substance. Obscure and equivocal though this term may be, at least it is not a protein which may be subject to the action of pepsin. So, the membrane's of the mucosa being indigestible, and certainly also impermeable for a molecule of such a large size as that of pepsin, will prevent the interior of the cells from being digested as long as the membrane is intact. But also it is scarcely probable that pepsin and acid are mixed in the secreting cells before being thrown out into the cavity of the stomach. Let us first consider the pepsin. Some authors believe that this enzyme is secreted in a preliminary inactive state, as a proenyzme, and only within the lumen of the stomach is converted to active pepsin. I do not feel quite sure that this is true. But in the second place, consider the acid. The essential substance which is secreted is the hydrogen ions. In consequence of the laws of electro-neutrality of any solution, the secretion of H-ions must be accompanied either by a simultaneous secretion of Cl-ions, or by an exchange with, e.g., the sodium ions of the chyme. We do not know yet which of these two processes really takes place nor do I believe even that this question has ever been asked at all. But whichever may be true of these two possibilities, if H^+ ions are to leave the glandular cells in the direction of the lumen of the stomach, the contents of these cells can never become acid, while secretion is taking place, but only more alkaline, or at least remain unchanged by absorbing the required amount of hydrogen ions from the great reservoir of buffers represented by the blood. So under no condition is the possibility of digestion of the living gastric cells presented. As soon as the cells become necrotic, digestion sets in, which fact is well-known in the etiology of gastric ulcer. Even for parasitic worms, recently Northrop

pointed out that dead worms are digestible, living worms are not. Northrop also showed that an ameba does not resist digestion if the enzyme has been injected into the living cell. The force which compels the hydrogen ions to flow in the direction of the lumen has been hitherto unknown, but it is not more mysterious than any other specific secretion of any gland.

The determination of the acidity of the gastric juice has played an important rôle from the beginning of scientific medicine and has undergone many changes in the course of the development of chemistry. I should like to attempt a representation of this field on the basis of modern physicochemical concepts. The gastric juice may be imitated by a simplified model consisting of a solution of peptone and of an excess of hydrochloric acid. Peptone means here only a mixture of protein compounds and products of their hydrolysis with the common property of amphoteric electrolytes due to the simultaneous presence of COOH- and NH_2-groups. An "excess" of hydrochloric acid means an amount of hydrochloric acid exceeding that amount which is necessary to form a salt with the peptone, a peptone-hydrochloride. We may call that amount of HCl which is used up to combine with the peptone to form a salt, the "combined HCl" and the excess of HCl the "free HCl." This is not the only possible definition of the much used terms, free and combined HCl, the sum of both representing the "total HCl." But with respect to the modern concepts, this definition seems to me the only one compatible with physical chemistry. Let us suppose there is given a solution of peptone and the equivalent amount of HCl, or in other words, a solution of pure peptone-hydrochloride; this solution is not neutral, but by hydrolysis is partially dissociated into free HCl and free peptone, thus showing an acid reaction and a certain amount of what we mght call "free hydrochloric acid." But there is a remarkable difference in the behavior of an excess of HCl and of that part of HCl which is the hydrolized part of a peptone-hydrochloride. In the first place, when there is an appreciable excess of HCl at all, the hydrolysis of the peptone-hydrochloride is almost abolished according to the mass action law, and practically all of the hydrogen ions are the H-ions fur-

nished by the excess of HCl. In the second place, when there is an excess of HCl, and when the solution is evaporated, the concentration of the H^+ ions will become greater and greater according to the progress of evaporation. There are some reagents which show color reaction only with a very great concentration

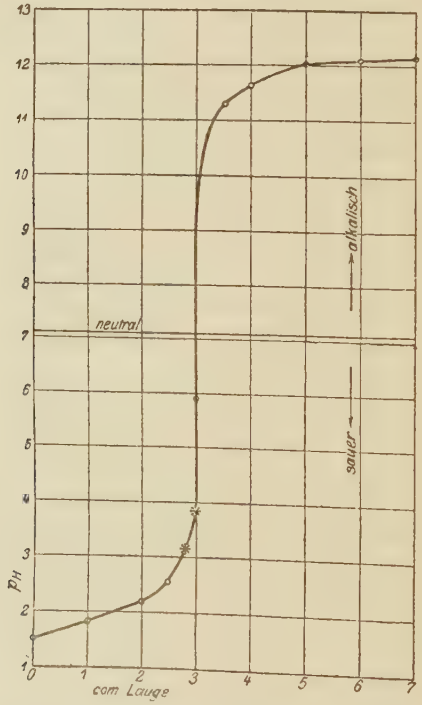

FIG. 1. Three cubic centimeters of 0.1 M HCl are being titrated with 0.1 M NaOH. Abscissa: cubic centimeter of NaOH added. Ordinate: pH.

of hydrogen ions, e.g., the Günzburg reagent, a mixture of phloruglucin and vanillin. Christiansen has shown that this reagent gives the well-known red color reaction with a strong HCl solution even at room temperature. Now, if a gastric juice contains an excess of HCl, this excess must reach, when the juice is being evaporated, finally such a high concentration as to be able to

react with the Günzburg reagent. When there is no excess of HCl but only an amount corresponding to the peptone, we have a solution of peptone-hydrochloride. According to a rule in physical chemistry, the hydrogen ion concentration produced by the hydrolysis of a salt consisting of a strong acid and a weak base,

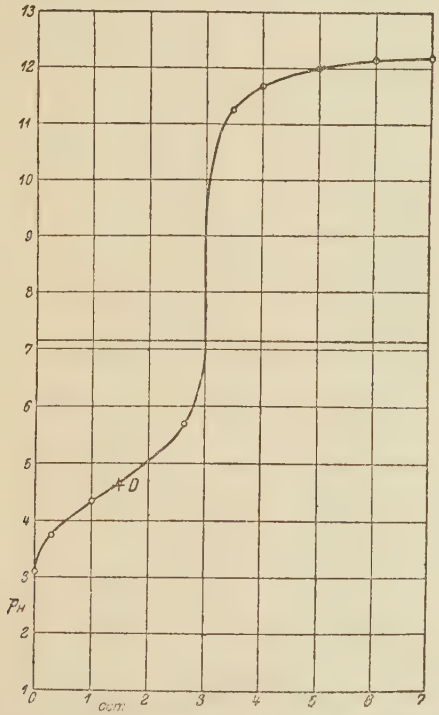

FIG. 2. Three cubic centimeters of 0.1 M acetic acid are being titrated with 0.1 M NaOH.

depends relatively little on the concentration of this salt. Therefore in evaporating the solution, the H^+ ion concentration sufficient to give the Günzburg test will not be attained.

In order to show the best way to obtain quantitative figures for the acidity we have to go back to the theory of titration curves. Let us begin with the titration of hydrochloric acid. A certain

amount of HCl, e.g., 3 cc. of a 0.1 M solution of HCl are titrated with a 0.1 M solution of NaOH. In figure 1 the abscissa gives the cubic centimeter of NaOH added to the acid, and the ordinate the pH produced by that. The curve obtained is called the titration curve of HCl. It is characterized by the fact that the pH is only slightly altered by the successive addition of NaOH and

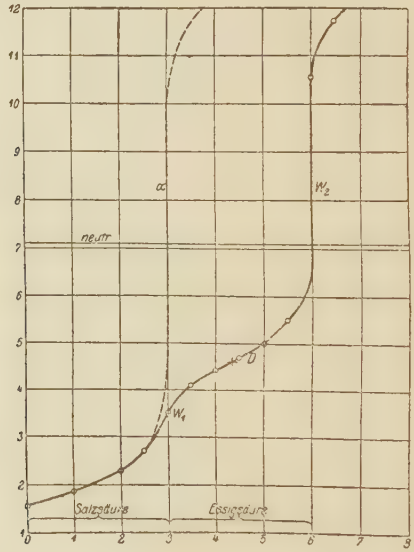

Fig. 3. A mixture of 3 cc. of 0.1 M HCl and of 3 cc. of 0.1 M acetic acid is being titrated with 0.1 M NaOH. W_1, W_2 the two inflection points of the titration curve. The dotted line α: the rise of the curve which would take place if no acetic acid were present.

only immediately before the point of neutralization the curve suddenly bends and goes up sharply. Here one drop of NaOH will cause a change in the pH from about 5 to 11. The drop causing the sudden rise of the curve represents the end-point of the titration.

Figure 2 shows the titration curve of a weaker acid such as acetic acid, titrated with NaOH as before. The pH in the be-

ginning is lower than in the case with HCl because acetic acid is less dissociated. A great part of the titration curve is represented by a straight line, the slope of which shows the buffer effect of the mixture of acetic acid and sodium acetate. When the point of neutralization is reached, the curve rises suddenly, but not quite to such an extent as in the case of HCl.

Now let us titrate a mixture of HCl and acetic acid (fig. 3).

FIG. 4. A mixture of 3 cc. of 0.1 M HCl acid and of 3 cc. of 0.1 M lactic acid is titrated with 0.1 M NaOH. The dotted line α has the same significance as in figure 3.

The titration curve begins in the same way as though there were only HCl present, because by an excess of HCl the dissociation of the weaker acid is completely suppressed. After the HCl has been used up by the titration, the curve begins rising, but now the buffer effect of the acetic acid begins; the rise is represented only by a slight bending upward of the curve; from here, the titration curve of the acetic acid follows. According to whether the second

acid is more or less weak, the slight bending at the end-point of HCl can be more or less distinguished. In acetic acid, as shown in this diagram, the bending can be seen fairly well. When we take lactic acid (fig. 4) instead of acetic acid for the second acid which is mixed with the HCl, the bend cannot be seen any longer, because lactic acid is much stronger than acetic acid. Now let us

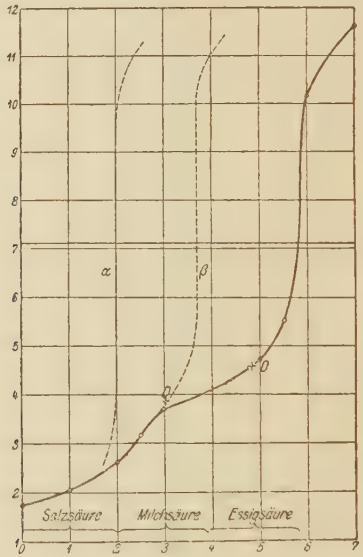

FIG. 5. A mixture of 3 cc. of 0.1 M HCl, 3 cc. 0.1 M lactic acid and 3 cc. 0.1 M acetic acid titrated with 0.1 M NaOH. α: rise of the curve, which really is suppressed by the buffer effect of lactic acid. β: rise of curve which really is suppressed by the buffer effect of the acetic acid.

consider how we can tell, from the titration curve, that another acid besides the HCl is present here. When we look at the pH at the beginning before adding any NaOH, this pH is due only to the HCl, because any weaker acid is virtually undissociated in the presence of an excess of HCl. We may assume with an accuracy sufficient for the present purpose, that the hydrogen ion concentration of an HCl solution is equal to the concentration

of HCl, or in other words, that HCl is dissociated completely, and that the activity of the hydrogen ions is equal to their concentration. Any deviation from this assumption is beyond the limits of error in the ordinary methods available for gastric juice. Therefore, from the pH at the beginning, we can calculate the concentration of free HCl, and we may construct the titration curve expected for a pure HCl solution of this concentration. The curve drawn according to this calculation is identical with

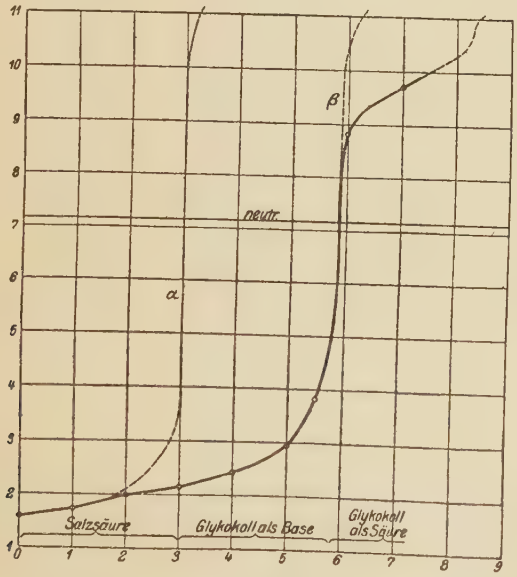

FIG. 6. Titration of HCl + glycocol.

the one obtained only in the beginning. At a certain point, a sudden rise should be expected as shown by the dotted lines, α, in figures 3 and 4. However, this rise does not take place, and the curve really obtained is smoothed by the second weak acid.

Figure 5 shows the titration curve for a mixture of HCl, lactic acid and acetic acid. The dotted line α shows how the curve would go if only HCl were present; the dotted line β shows how much further it would go if HCl and lactic acid would be present,

and the whole curve shows the real course in the presence of all three acids.[2] Any point of inflection, as boundary between the different acids, is more and more abolished. There results only an almost straight-line curve.

The hydrochloride of a weak base interferes with the titration

Fig. 7. Titration curve of a gastric juice after an Ewald test meal. The ordinate "rot" means diaminoazobenzene is red. The ordinate "lachsf." means diaminoazobenzene is salmon pink. The ordinate "citr. gelb." means diaminoazobenzene is lemon yellow. The ordinate "Phenolpht." means phenolphthalein turning pink. The dotted line shows how the curve would rise if the acidity would be produced only by the hydrochloric acid the amount of which can be calculated from the initial pH.

curve in the same way as a weak acid, and there is no means of distinguishing whether HCl is mixed with a weak acid such as acetic acid, or with the hydrochloride of a weak base, such as ammonia. An amphoteric electrolyte behaves, according to the

[2] The dotted part γ has no significance in these considerations and should be neglected by the reader.

pH range, sometimes as a weak base, and sometimes as a weak acid. In figure 6, glycocol shows its smoothing effect on the titration curve of HCl first at an acid reaction, preventing the rise of the curve as expected in α_1 and further at an alkaline reaction, preventing the expected rising of the curve at β.

Now we are prepared to apply these results to the gastric juice. This may be regarded in a somewhat simplified way as a mixture

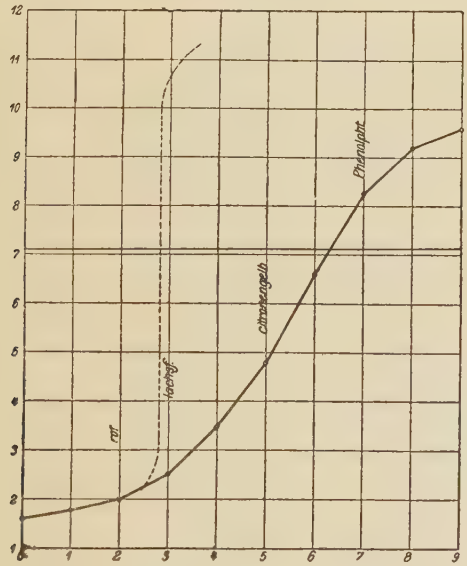

Fig. 8. Titration curve of another gastric juice.

of HCl and the hydrochloride of a mixture of different proteins, peptones, peptides, of very different dissociation constants, and each of them possessing not only one or two, but even several successive dissociation constants. It may be realized now, that the effect of all these different successive constants on the titration curve is simply a smoothing of the curve to an almost rectilinear shape, as shown in figures 7, 8 and 9. This is the general shape of the titration curve in gastric juice, as you can

see from different examples (figs. 8, 9, 10). The magnitudes which characterize this curve are the following:

1. The pH of the juice in the original state before the addition of any sodium hydroxide. The H-ion concentration corresponding to this pH is equal to the concentration of the "free hydrochloric acid."

2. The point of the curve corresponding to that amount of NaOH which just neutralizes the free hydrochloric acid. A com-

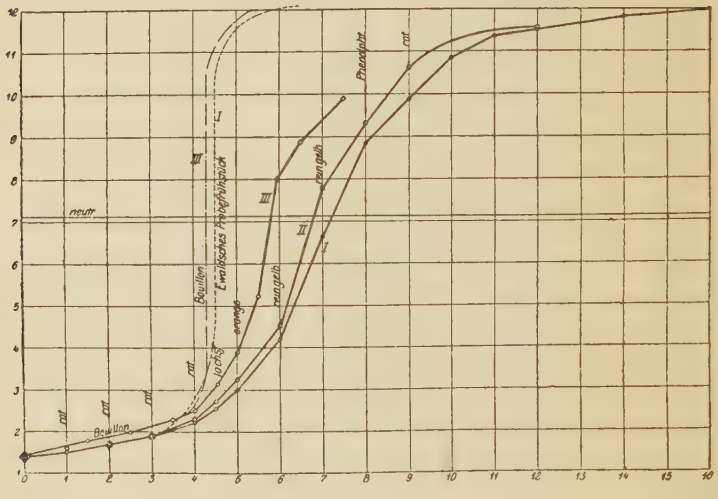

Fig. 9. Curve I: Titration curve of a gastric juice after Ewald test meal. Curve II: The same juice after removing the phosphates. Curve III: Gastric juice of the same person after a test meal of plain broth.

parative experience in titration curves of many gastric juices shows that this point is reached at a pretty well defined pH which is practically the same for any gastric juice within the limits of error. This pH is about 2.8, corresponding to a rather strong acid reaction. That means that the hydrochlorides of the peptones are strongly hydrolyzed. We have just mentioned the fact that the hydrogen ion concentration produced by the hydrolysis of a hydrolyzable salt depends only little on the concentration

of this salt. Hence it follows that almost independent of the amount of peptones in the gastric juice within certain reasonable limits, the hydrogen ion concentration, at that point of the titration curve where the free hydrochloric acid is neutralized, will be approximately the same, the pH amounting to the value mentioned above, 2.8, as shown by experience. In this fact a method is involved for the titration of the hydrogen ions of the gastric juice. This is remarkable and somewhat contradictory to the general assumption that hydrogen ion concentration cannot be determined by a chemical method at all, but requires a physical method, such as the gas chain which does not disturb the equilibrium of the ions in the solution. It is only due to the particular behavior of the gastric juice and only approximately true that the hydrogen ion concentration can be determined by titration. This method would consist in the titration by means of an indicator which just indicates the pH 2.8, taking into consideration all the errors of an indicator due to the relatively high concentration of proteins and peptones. Theoretical consideration showed that only a basic indicator can fulfill this condition. I think there is only one indicator available, the above mentioned Toepffer reagent, diamino-azobenzene. The point where the red color of this indicator is just turning to a salmon pink is the right one for the purpose and not the orange or yellow shade usually reached, a point having absolutely no significance. The salmon pink point is not very sharp, it is true, and therefore this method has not been introduced generally into the routine laboratory and other methods with sharper end-points are used instead. But fortunately a sharp end-point is of little use, as for the needs of the clinic the accuracy need not be very high. An error of 10 per cent is of no importance at all, as in pathological cases the variations from the normal are much larger and even the limits of the physiological range are greater. In fact, in chemistry it may sometimes happen that an analytical method furnishing very accurate numbers has no meaning at all, whereas another method giving less sharp results has a useful meaning and is preferable. For instance, the determining of the free HCl with an iodometric titration such as Sahli recommended, gives astonishingly sharp end-points,

but the obtained numbers are not the desired values for free hydrochloric acid.

The fact that the titration curve of the gastric juice is approximately a straight line from pH 2.8 to alkaline reaction, enables us to obtain all the data characteristic of a specimen of gastric juice by titrations. I will show that by an example. In figure 7 gastric juice has been titrated with 0.1 M NaOH. The abscissa shows the number of cubic centimeters of NaOH added to 10 cc. of the gastric juice; the ordinate gives the pH as measured by the gas chain. Now, we add at the beginning some drops of diaminoazobenzene and of phenolphthalein. The pH of the juice at the beginning is 1.5, or, the hydrogen ion concentration is 0.032 molar, or also, the content of free HCl according to our definition is 0.032 molar. Ten cubic centimeters of the juice should require 3.2 cc. of the NaOH to neutralize the free HCl. At this point, Toepffer reagent should be salmon pink, and the pH approximately 2.8. That is the case, as you see in figures 7 or 8. The dotted line shows how the titration curve would go if no other substance besides the free HCl acid were present. Instead the titration curve shows a straight linear course, the beginning of which may just be recognized as a little wave, but it is rare for this wave to be apparent at all. Now we continue titrating until the Toepffer reagent has reached its definite pure yellow shade. This corresponds in the gastric juice to a pH of 6.0, as control measurements have shown in many cases. We continue titrating until the phenolphthalein just turns pink. This point corresponds to a pH of 8. So we are able to draw the whole titration curve from the titration to the three points: Toepffer reagent salmon pink, Toepffer reagent yellow, phenolphthalein pink. The first point is virtually identical with the free hydrochloric acid. The middle point corresponds to neutral reaction and may be considered as the titration point for total acid, if this concept has a clear meaning at all. The difference between free and total acid may be called combined acid.

All of these concepts are not very sharply defined, as you see, and represent somewhat of a compromise between the usual terms of earlier periods and physicochemical concepts. The reason why

we should adhere to these concepts though they cannot be defined in a really adequate way, is for their clinical significance.

The free acid, or the hydrogen ion concentration, shows in how far the acidity of the gastric juice is sufficient to activate the pepsin for its proteolytic power. The pH is a direct measure of the digestive efficiency of the gastric juice. The total acid gives a completely different thing. Provided there is no appreciable amount of lactic acid or other organic acids, the total acid repre-

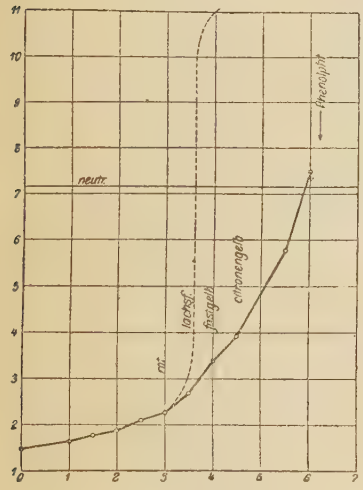

Fig. 10.

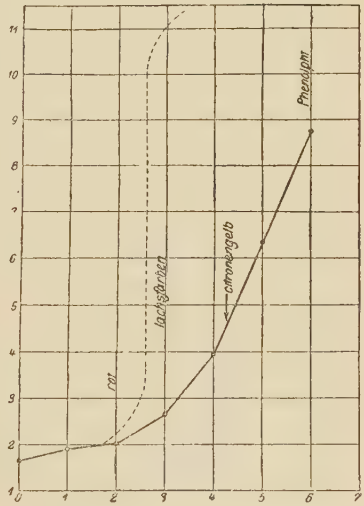

Fig. 11.

Figs. 10 and 11. Two cases of normal gastric juice after Ewald test meal.

sents the amount of acid which the gastric glands have secreted. The acid may have been in part neutralized after the secretion in the lumen of the stomach by proteins or peptones of the food, or by protein products resulting from some pathological processes, such as pus or mucus or other ulcerous secretions. Therefore the total acid shows the activity or efficiency of the gastric glands in secreting acid. It may happen that the intended effect of the acid is abolished by the secondary effect of neutralization but the

glands *were* able to secrete the acid. It is of great diagnostic value to distinguish an anacidity which is due to a lack of glandular activity, e.g., in atrophy of the glands, or an anacidity which is due to the fact that the secretion of the glands is normal but annihilated by a subsequent process of neutralization.

Let us consider now some titration curves of the gastric juice

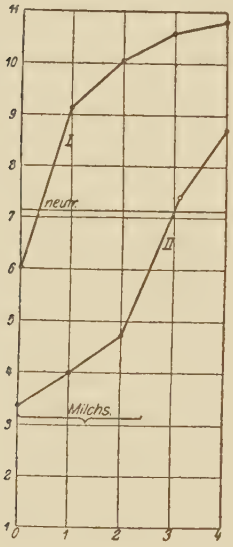

Fig. 12.

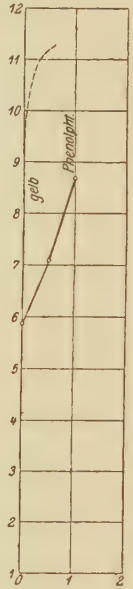

Fig. 13.

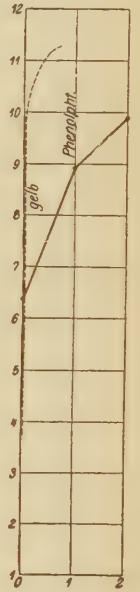

Fig. 14.

Figs. 12, 13, 14. Three titration curves of hypoacidity after Ewald test meal. In figure 12, curve II is the same juice as in curve I after addition of lactic acid to the gastric juice.

from this point of view. Figures 7, 8, 10 and 11 show normal gastric juices after an Ewald test meal. Figure 9, curve I shows likewise the titration curve of a gastric juice after an Ewald test meal. The dotted line I shows how the curve would go up if the free hydrochloric acid, as recognized from the pH at the beginning of the titration, were the only substance combining with the NaOH. Curve II has the following meaning. As there are also phosphates

in the food and in liquids of the body, it could be asked in how far the phosphates interfere with the simplified interpretation of the titration curve. In a sample of this gastric juice the phosphates were removed by precipitation with some calcium at alkaline reaction, the pH of the filtrate was restituted to the original value by addition of the suitable amount of HCl, and the titration carried out once more. As you see from curve II in figure 9, the

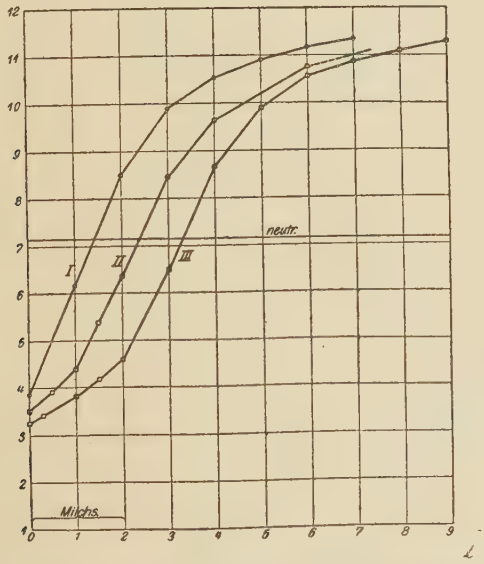

Fig. 15. A case of anacidity, curves II and III after addition of lactic acid.

difference from curve I is negligible, so that really the phosphates need not be taken into consideration. But the curve shows another very instructive experiment. In the same patient, instead of an Ewald test meal containing some bread, a test meal was administered consisting of only a little broth, poor in protein or base-combining substances. The pH of the gastric juice is exactly the same as before, or, the free hydrochloric acid is the same, but the slope of the titration curve is much steeper (fig. 9,

curve III), or the combined acid is less. From this you may infer that under normal conditions the gastric secretion aims at reaching a definite pH, and that the amount of secreted hydrochloric acid necessary for this purpose differs according to acid-combining capacity of the food. This is an approximation, of course, neglecting the fact that there are substances which stimulate the secretion, and others, like fats, which diminish the secretion of gastric juice. Such secondary influences, however, cannot obscure the general law regulating the secretion, which may be expressed as follows: The normal tendency is to secrete such an amount of HCl as to reach an approximately defined pH, but not a defined total amount of acid.

Figures 12 to 15 show some titration curves in hypo- and anacidities. On the left, figure 12, is a gastric juice without any acid, pH amounting to 6. The curve is simply the right hand end of an ordinary titration curve of a normal gastric juice. Curve II, figure 12, shows the same juice after the addition of a very large amount of lactic acid. The pH is much lowered, reaching 3.4. And still, you know that even with this very great amount of lactic acid, the pH will never reach 2.8, and will never induce us to assume the presence of any free hydrochloric acid. Figure 13 shows the titration curve of a case of anacidity, with an initial pH of 6, and the right hand figure is about the same. Figure 15, curve I, shows a case of subacidity, but with a pH of about 4. This juice contains, as we may say, no free, but combined hydrochloric acid. The curves II and III show the same juice after addition of large amounts of lactic acid. You may see, that the initial point, though little changed, never reaches the characteristic point, 2.8.

Table 1 shows a collection of titrations of gastric juices after a test meal. The numbers mean the cubic centimeters of 0.1 N NaOH, as would be used by 100 cc. of the gastric juice. Here are the values for free and total HCl, cases of hyperacidities, normal acidity, hypoacidities and achylias. The last column gives figures for a comparative determination of the rennet.

Herewith we are entering the chapter on gastric enzymes. There is only a slight variation of the rennet content in hyper-

acidities and normal cases, but there is certainly a parallelism of acid and rennet in any case of insufficient acidity. I should like

TABLE 1

NUMBER		TITRATION WITH DIMETHYLAMIDOAZOBENZOL*			FREE HCl	TOTAL HCl	RENNET CONTENT FIRST DAY
		a	b	c			
1	Catarrhal jaundice	58	68	94	58	81	160
2	Hyperacidities	58	66	92	58	79	150
3	Hyperacidities	62	70	79	62	75	160
4	Hyperacidities	36	45	80	36	63	160
5	Hyperacidities	38	47	77	38	62	96
6	Hyperacidities	32	42	64	32	53	150
7	Hyperacidities	38	47	67	38	57	190
8	Normal acidities	32	40	50	32	45	100
9	Normal acidities	18	32	58	18	45	100
10	Normal acidities	30	38	52	30	45	160
11	Normal acidities	18	30	58	18	44	120
12	Normal acidities	29	37	45	29	41	125
13	Normal acidities	25	33	50	25	41	160
14	Normal acidities	29	32	39	29	41	100
15	Normal acidities	18	27	43	18	35	110
16	Catarrhal jaundice	22	28	42	22	35	120
17	Hypoacidities	18	26	32	18	29	120
18	Hypoacidities	6	12	32	6	22	150
19	Hypoacidities	3	10	30	3	20	100
20	Hypoacidities	0	8	20	0	14	50
21	Hypoacidities	0	4	17	0	11	20
22	Hypoacidities	−4	+4	15	0	10	30
23	Hypoacidities	−14	−11	+12	0	0	30
24	Achylia	0	3	6	0	4.5	4
25	Achylia	−14	−8	+6	0	0	1.5
26	Achylia	−19	−13	+34	0	0	6.5
27	Achylia				0	0	6
28	Achylia	−2	0	+6	0	3	3

* a. Salmon pink. b. Pure yellow. c. Phenolphthalein end-point.

to emphasize the fact that the rennet content is approximately proportional to the total acid, but not to the free acid. There are large differences in the rennet content in gastric juices in all of

which there is no free acid at all. But the amount of total acid is rather parallel to the rennet content. That shows again that the amount of total acid is the reasonable scale for the faculty of secretion. The determination of rennet can be made in a very accurate way. Many errors are possible and have really been made; however, a very reliable method is available. This method is very simple and of the greatest practical importance whenever a deficiency of the gastric secretion is to be considered for diagnosis. Great differences in the secretory power of the stomach can be easily and quantitatively detected from the rennet determinations. But what is the significance of this rennet?

With respect to the rennet, we have to deal with the problems of the conditions under which the rennet functions, the chemistry of its activity and the physiological significance of its action. The necessary conditions for the action of rennet upon casein are the presence of a soluble calcium salt and a pH within a certain definite range. The optimum pH is about 6 to 6.5. This is a very astonishing fact because a pH of 6 is never present or at least never maintained for an appreciable period in the gastric juice of an adult, where great amounts of rennet are present through all stages of digestion without the conditions for its action. In the stomach of the infant, the conditions for rennet are better. The pH of woman's milk is about 6.5, close to the optimum condition for the rennet effect. The pH in the stomach of the infant reaches about 5, and certainly in the course of the digestion there will be a considerable time during which the pH is just the optimum for rennet. But in the adult, the effect of rennet cannot be recognized at all. When the acidity becomes higher, the coagulation of milk will be produced by the acid alone. The optimum for the acid coagulation of milk is 4.7, the isoelectric point of the casein. Under the conditions given in milk, with its high concentration in casein and in calcium, the acid optimum for casein coagulation is not very sharp, and any appreciable acid reaction will coagulate the milk even without rennet. Therefore, in the gastric juice of normal acidity the significance of rennet cannot be understood at all. This fact may convey us to the conclusion, that rennet, in the adult, has no significance but is only an in-

evitable by-product of gastric secretion, or even more, that rennet is not a definite enzyme at all and that the apparent effect of it is in reality the effect of pepsin under different conditions. Pepsin may be a hydrolytic enzyme at a pH of 1 to 2 and at a pH of 6 what we call rennet. An enormous discussion has taken place and been renewed again and again between the unitarians, who assume that pepsin and rennet are identical, and their adversaries. Most of the experiments in which an attempt was made to separate pepsin from rennet meet serious objections when regarded in the light of modern physicochemical views. Apparently in only one kind of experiment of Hammarsten, published recently, was success attained in preparing a rennet free from pepsin. However, this is the rennet of the calf, not of an adult animal. For a long time, a certain difference between the rennet of infants and adults has been asserted, and I. Bang even distinguished the real chymosin of the infant from the parachymosin of the adult. Referring to the recent investigation of Hammarsten, the existence of a rennet which can be isolated from pepsin, appears probable for calf's rennet, but the same is not yet proven for the rennet of the adult man or animal. For pig's rennet and pepsin, Rothstein and I pointed out a simultaneous destruction by means of different agents, such as alkali or heat, some kinds of adsorption and others.

The sensitivity of rennet or pepsin for alkali is very remarkable. Alkali destroys rennet rapidly and irreversibly. Pig's rennet, which has been investigated by Rothstein and myself, is virtually stable for any period when kept at acid reaction. Even at such a weak acid reaction as that which corresponds to the optimum effect of rennet, pH = 6, the stability of rennet is fairly good. From this point, any slight increase of the alkalinity increases the speed of the self-destruction of rennet to an enormous degree. It can be shown that the irreversible and definite destruction of rennet takes place at a rate which is about proportional to the 4th power of the hydroxyl ion concentration. The influence of the temperature upon this destruction is very remarkable. The alkalinity of a solution may either be described in terms of hydrogen or of hydroxyl ion concentration. As the ion product of

water varies strongly with the temperature, two solutions of different temperatures and with equal hydrogen ion concentration will have different hydroxyl ion concentrations and vice versa. When the alkalinity of the solution is expressed in terms of hydroxyl ion concentration, the speed of destruction of rennet is independent of temperature within a wide range. But when the alkalinity is

TABLE 2

NUMBER	ACIDITY				RENNET		PEPSIN	
	a	b	c		Units	Per cent of normal average	Units	Per cent of normal average
1	46	57	86	Superacidities	156	125	16.2	108
2	23	32	42	Normal acidities	160	128	12.5	83
3	24	34	52	Normal acidities	140	112	18.0	120
4	27	36	52	Normal acidities	127	102	16.0	107
5	34	44	58	Normal acidities	120	98	13.5	90
6	15	24	36	Normal acidities	125	100	14.0	93
7	14	27	55	Normal acidities	125	100	16.0	107
8	16	25	38	Normal acidities	75	60	6.0	40
9	7	18	39	Hypoacidities	68	54	7.5	50
10	7	18	49	Hypoacidities	40	32	3.9	26
11	0	6	26	Profound acidities	87	62	5.8	39
12	−8	+4	10	Profound acidities	50	40	2.2	15
13	?	0	10	Profound acidities	7.5	4	0	0
14	−17	+3	8	Profound acidities	1.5	1.2	0	0
15	−8	0	+8	Profound acidities	1.5	1.2	0	0
16	−8	0	+7	Achylia	4	3.2	0	0

described in terms of hydrogen ions, the destruction of rennet has a great temperature coefficient. A solution with a pOH of 7 destroys the rennet with the same speed, whether it works at 18 or at 40°. But a solution with a pH of 7 destroys the rennet some hundred times as fast at 37° as it does at 18°. The destruction of pepsin was always absolutely parallel to that of rennet in our experiments.

In this respect it is interesting also to see that the ratio of rennet to pepsin in the gastric juice in different normal and pathological cases is always the same within the limits of error. In table 2 a comparative determination of rennet and pepsin is shown for a series of different cases. The units for pepsin and for rennet are arbitrary, of course, and refer to a stable arbitrary stock solution of the enzymes. The method of determination of rennet consisted in finding out that dilution of the gastric juice which caused coagulation of diluted milk in the same time as the arbitrary but, once for all, fixed amount of the comparative standard rennet solution, all conditions being accurately defined: temperature, total volume, calcium content, the manner of moving or stirring the solution, etc. By this method, the comparative determination of rennet can be carried out within an accuracy of at least 5 per cent of the total value. The method of determination of *pepsin* was based on the fact that sulfosalicylic acid is such a strong acid that it activates the pepsin like HCl; and at the same time this acid is a well-known protein reagent. When a solution of a protein such as some diluted serum is mixed with sulfosalicylic acid, a milky turbidity is obtained which under certain conditions gives a rather stable opaque colloidal suspension. The action of pepsin is recognized as this turbid solution becomes more and more transparent. The method of determination of pepsin consists in finding out, by a series experiment, that concentration of the gastric juice which brings about the same speed of the disappearance of the turbidity as an arbitrary standard unit of pepsin does. The observation should not be referred to the time necessary to bring about the complete transparency of the solution; this is a very unreliable limit because it is asymptotic. The observation should be directed to the *course* of the clearing. This method is easy and fairly accurate, though not quite so sensitive as the method for rennet. That is why in some cases a very small amount of rennet is found (e.g., 1 per cent of the normal average value), while the amount of pepsin is simply designated = 0. Among all these cases there is only one in which the amount of rennet and pepsin, referred to the normal average amounts, is significantly different, and I hesitate to

accept such a single exceptional observation, though it is my own observation.

Concerning the chemistry of the rennet effect, it has been known since the investigations of Hammarsten, that the presence of a soluble calcium salt is necessary for the coagulation of casein, but that a chemical change of the casein is produced by rennet even in the absence of calcium. Rennet is supposed to change the casein into paracasein, and paracasein is supposed to be coagulated by calcium. Among the great variety of investigations aiming at the explanation of the chemical nature of the rennet effect, I should like to give a short report on some studies by Dr. Marui in my laboratory in Japan. Hammarsten had pointed out that the presence of calcium is indispensable for the rennet effect. It is known also that boiled milk is much less subject to coagulation by rennet and as a rule cannot be coagulated by rennet at all unless an excess of a calcium salt is added. This fact seems to suggest that the calcium, which is dissolved in milk partially as a free simple calcium salt, is combined with the casein when heated and withdrawn from the solution. However, it could be shown that this is not true. The method was compensation dialysis which had been previously applied by Rona and myself for proving that the blood sugar is present in the blood in a free dialyzable condition. For the present purpose the method consists in finding by trial that concentration of $CaCl_2$ in an aqueous solution which is in diffusion equilibrium when separated from the milk by means of an ordinary collodion membrane which is impermeable only for colloids. This method has shown that about two-thirds or a little less of the calcium is present in a free diffusible condition and that this amount is not appreciably altered by a previous heating of the milk. So any combination of calcium with a colloidal compound by the heating, which might exceed that combination before heating, cannot be proved. On the other hand, it has been shown that an artificial solution of casein containing no calcium at all, undergoes an alteration by heating so that after cooling and on addition of calcium and rennet the coagulation will be suppressed. The ability of calcium to bring about coagulation when a given amount of calcium chloride and

rennet is subsequently added to a solution of casein depends largely on the temperature at which the casein has been dissolved before the addition of the calcium and the rennet. Even such a small difference of temperature in the dissolving process (fig. 16) as from 20° to 25°C. has a remarkable preventive effect on the velocity of rennet coagulation. One would suspect that this change of the casein is somewhat similar to what is called denaturization in albumins and globulins. However, this comparison

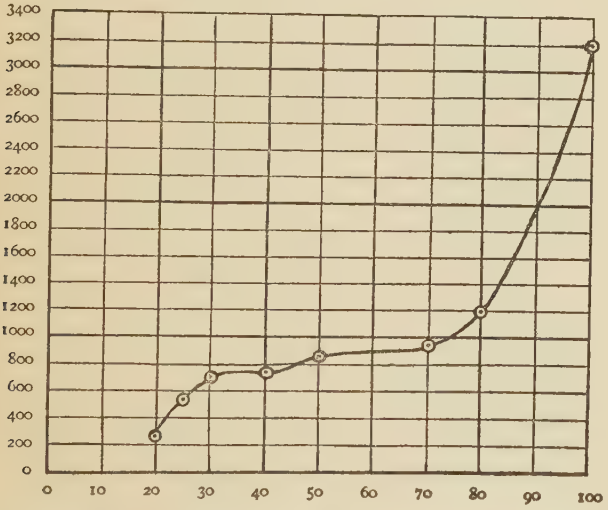

FIG. 16. Abscissa: Temperature at which the casein had been dissolved in sodium acetate. Ordinate: Period of time after which the addition of a constant amount of rennet and $CaCl_2$ brought about coagulation at 19°C.

is not quite adequate. For the denaturization of albumin is a completely irreversible process, while the casein regains its original properties when precipitated from the solution by acid, washed and completely dried at low temperature. So we are forced to the assumption that the alteration of casein solution by heating is a change of the colloidal condition. That means, after all, we do not know yet what it really is. After all I should like to emphasize that even strongly heated casein can be coagulated by

rennet provided a sufficiently large amount of any calcium salt is added.

Another part of these investigations dealt with the question of whether the calcium salts can be replaced by other metallic salts. The result was, in the first place, that calcium cannot be replaced by any monovalent metals even in high concentration. However, bivalent metals show a behavior which in general is the same and may be described as follows. In the absence of rennet the salts of bivalent metals have an effect on a casein solution depending on the concentration. A sufficiently great concentration of the salt will precipitate the casein, a low concentration will not. In some salts, especially in trivalent cations, there is a zone of very high concentration which does not precipitate the casein, a somewhat lower concentration zone which does precipitate and a still lower concentration zone which does not. That is what is called the "irregular precipitation series" in colloidal chemistry.

The effect of rennet consists here in producing a precipitation somewhere within those concentration zones of the salts which do not precipitate the casein by themselves. The exceptional position of Ca among the other bivalent metals is only that the zone of calcium concentration which does not give a precipitation without rennet and does with rennet is relatively wide. Ba and Sr behave almost like Ca. Also in Mg there is a certain zone of concentration in which it activates the rennet effect. This zone is much smaller, and the effect of magnesium is the more interesting therefore.

The physicochemical significance of the coagulation process produced by the rennet has been the object of another study. The result may be summarized as follows. Casein acts as a protective colloid. This means that other colloidal solutions, which are not stable by themselves, can be made stable by the presence of casein, in the same way as they can be made stable by gelatin and other protein or colloidal carbohydrates. Rennet causes some alteration of casein which deprives it of its protective action. In milk it is the fat globules and the partially insoluble phosphates of calcium and magnesium which cause the formation of a stable colloidal solution by the protective effect of casein. The casein

forms a thin superficial layer around each fat drop, thus preventing them from coagulating or agglutinating, or from flowing together. Now, Ca salts have a great flocculating power on electrically negatively charged colloids. Enough soluble calcium salts are present in milk to coagulate all kinds of suspended particles, were they not protected by casein. Rennet destroys the protecting power of casein. This can be proven by some experiments with different colloids protected by casein. For instance, when a precipitation of calcium phosphate or even calcium oxalate is brought about in an aqueous solution in the presence of a small amount of casein, the calcium phosphate or oxalate will form a colloidal solution looking just like milk, though not containing any fat. Such a solution can be coagulated by rennet like natural milk. Or let us take a colloidal solution of gum mastic. Such a solution, produced by diluting the true alcoholic solution of mastic with much water, forms a turbid milky fluid which can be coagulated by small amounts of calcium salts. When a little casein is present, the calcium will not cause a flocculation. If rennet is now added, the protective power of the traces of casein is abolished and the whole of the mastic is coagulated, furnishing a precipitate exactly like cheese, this cheese consisting of gum mastic. So in general, the visible effect of rennet may be considered as the abolition of the protecting capacity of casein exerted on a colloidal solution. Rennet coagulates any colloidal solution the stability of which is due to the protective effect of casein.

The physiological significance of the rennet is still somewhat doubtful. One could surmise that the coagulation of the casein might be a preparation for the digestion either by pepsin or trypsin. But that is certainly not true. Nothing can be more easily digested by both trypsin or pepsin than casein homogeneously dissolved in a medium of adequate pH. Another supposition is that the coagulation of the casein is to prevent the undigested casein from being absorbed by the stomach or the intestine. As it is known that genuine protein substances, even when administered per os, provided the quantity is large enough, act as antigens and produce specific precipitins, it may be deduced that

the physiological purpose of the coagulation of the casein is to prevent the absorption of an antigenic protein. In fact, though a precipitin for cow's casein can easily be obtained by injection of casein in a rabbit, the occurrence of a precipitin in the blood of an infant after feeding with cow's milk has never been described, as far as I know.

Proceeding now to the pepsin, both the physicochemical significance and the conditions of its activity are much better known. The fact that the hydrolysis of the proteins does not go so far as to simple dipeptides, and the increase of free carboxyl or amino groups cannot be detected as easily as in the tryptic digestion, had been interpreted by the assumption that pepsin does not act upon the peptic linkages of the protein molecules. However, recently Waldschmidt-Leitz emphasized the fact that, on application of a suitable technique and suitable proteins also in the peptic digestion, an equal number of carboxyl and amino groups arise in the same way as they do with trypsin. The difference between pepsin and trypsin does not seem to consist in the difference of the linkages which are opened by the enzyme, but only in the fact that pepsin has a specific affinity for the more complex proteins. For in general, the specific affinity of an enzyme not only depends on the atomic group which is to be hydrolyzed, but also on the configuration of the whole molecule. This has been known, for instance, in the carbohydrate-splitting enzymes for a very long time. Concerning the pH optimum for peptic digestion, the original statement of the pH optimum being 1.7 should be corrected a little. The pH optimum does not depend on the physicochemical properties of the enzyme alone, but also a little on those of the substrate. Northrop has shown that the pH optimum for peptic digestion depends on the isoelectric point of the protein which is to be digested. The pH optimum for the digestion of casein is 1.8, that of gelatin or globin is 2.2. At pH 5 there is virtually no trace of digestion to be observed with casein or gelatin, while hemoglobin at this pH is digested by pepsin, though slightly. But in any case the range of the pH optimum for the peptic digestion of different proteins is so small that practically the optimum for pepsin, for any proteolytic digestion, coin-

cides with the acidity of the normal gastric juice of the adult, while the normal acidity of the infant is always far removed from any pH which might activate the pepsin.

Finally there is an enzyme in the gastric juice of a very doubtful significance,—the lipase. A long time was required to prove the existence of this enzyme and even today some authors may claim that the lipase which may be found in the gastric juice is due to regurgitation of pancreatic juice. The efficiency of the gastric lipase is so small that it can be studied only by means of sensitive methods. The easiest method is one described by Rona and myself which determines the hydrolysis of a saturated solution of tributyrin by the measurement of the surface tension, which method has also been adopted by Willstätter in his studies on lipase. With this method, the presence of a lipase can be detected in any normal gastric juice, even without any trypsin being present. This fact shows that this lipase is not due to the regurgitation of intestinal juice. Furthermore, Davidsohn showed that the properties of the gastric and the pancreatic lipase are different from each other. Sometimes pancreatic lipase can be found also in the gastric juice, as in the case of regurgitation, and it can be distinguished by the difference of the pH optimum of its activity. The pH optimum for lipase of the pancreas and likewise of the blood serum is an alkaline reaction at a pH of about 8 or still greater, whereas the optimum for the gastric lipase is at a pH of about 4.5, corresponding to the acidity of the infant's gastric juice. To distinguish the lipase of the stomach from the pancreas it is sufficient to carry out two parallel experiments with tributyrin, one with the addition of some primary phosphate, and the other with the addition of some secondary phosphate. Gastric lipase works much quicker in the primary phosphate; intestinal lipase works quicker with the secondary phosphate. The significance of these tests has become a little doubtful since Willstätter has shown that the pH optimum for lipase depends on some accompanying impurities. Willstätter prepared a lipase by the extraction with glycerin of the acetone-treated mucosa of pig's stomach. He pointed out that the pH optimum in the impure enzyme is at a somewhat acid reaction,

such as described by Davidsohn, but that with the progress of the purification of the enzyme, the optimum shifts more and more to the alkaline side and can no longer be distinguished from the one for pancreatic lipase. It may be that the differences between the gastric and the pancreatic lipase are due to the different kinds of impurities and the enzymes are perhaps identical. But in any case the lipase secreted by the pancreas can still be distinguished, by this method, from the lipase secreted by the stomach. In any case, Willstätter has shown decisively that lipase may originate in the stomach by the fact that he succeeded in extracting a lipase from the mucosa of the stomach. I should like to emphasize another observation in this respect. In some cases of complete achylia in pernicious anemia, I found a complete lack of the lipase in the gastric juice accompanied, of course, by a complete lack of pepsin and rennet also. As there is no doubt that there was an approximately normal pancreatic digestion in these cases, the specificity of the gastric lipase can be proved by such observations.

I mentioned the fact that it is almost impossible to separate pepsin and rennet. It is a different thing with lipase. None of the usual commercial pepsin preparations contains any trace of the gastric lipase, so the pepsin can be separated very easily from the lipase. The significance of the gastric lipase is obscure. Even in infants, where the pH of the gastric juice is most adequate for the action of the lipase, no appreciable digestion of fats in the stomach seems to take place. A further study of the gastric lipase seems to be worth while. For the clinicians it may be of value for diagnosis which has not yet been utilized.

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ANALYSIS OF THE ACTION POTENTIAL IN NERVE[1]

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Based on Experiments Done in Collaboration with H. S. Gasser and G. H. Bishop

THE aim of the physiologist as a scientist is to ascertain the mechanism of living things. Certain of the problems with which he has had to deal have quickly yielded to his efforts, others have been only partially solved, and there are still many concerning which our information is practically nil. This difficulty in the way of extending physiological knowledge is a double one, namely, inability to grasp the relations of things and lack of adequate methods of investigation. The problem with which this paper deals, namely, the action potential in nerve, is one of those that has failed of solution mainly because the tools available have been inadequate.

Though it was realized by the ancients that the nerves serve to put the parts of the body into connection with the brain and spinal cord and though, in more recent times, it was surmised that this is accomplished through the transmission of something vaguely called the nerve impulse, it was only in 1843 that physiologists succeeded in finding any objective evidences of activity that might serve as a means of ascertaining what is transpiring in a conducting nerve. In that year du Bois Reymond discovered that an active point on a nerve becomes negative electrically with respect to any inactive part. He suspected at the time that the effect was due to repeated waves of negative potential travelling along the nerve from the point artificially stimulated; but the galvanometers then available, on account of the relatively great mass of their moving parts, responded so slowly that direct analysis of the deflection produced by nerve potentials was impossible.

[1] Lecture delivered February 19, 1927.

A little later, through the use of a very ingenious expedient, the differential rheotome, Bernstein succeeded in proving that the deflection of the galvanometer actually is produced by discrete waves of negative potential; but time has shown that even the rheotome is incapable of revealing the true form of the action potential wave.

Further progress awaited improvement in electrical methods. The capillary electrometer, an interesting recording instrument that has been so well used since 1898, particularly by the English group of physiologists, has contributed relatively little to the particular phase of nerve physiology with which this paper deals. Despite the fact that it is almost infinitely quicker than the types of galvanometers previously available, it still apparently is too slow for the problem in hand. And the same statement may be made with regard to the string galvanometer in the form in which it has thus far been employed.

For some time, however, physicists have had at their command a device for recording changes in electrical potential that is sufficiently quick for every purpose, namely, the cathode ray oscillograph. This instrument, described by Braun in 1897, consists, in a word, of an evacuated tube (*B*, fig. 1), through which a fine stream of electrons is driven against a fluorescent screen, producing a spot of light at the point of impact (*X*). This electron stream can be deflected from its straight course through the tube by applying an electrical field across its path and the resulting movement of the spot of light can be followed with the eye or photographed. Since the mass of the moving part of the mechanism, namely, the electron stream, practically is negligible, there is scarcely any limit to the rate of potential change that can be recorded. The capabilities of the Braun tube in this sense may be illustrated by a record (fig. 2) made by the French physicist, Dufour, picturing a potential oscillating more than 200,000,000 times a second. Obviously, as regards quickness the oscillograph leaves nothing to be desired. But quickness is not the only requisite; to be satisfactory the instrument must also be sufficiently sensitive. The action potential of nerve may not exceed a few thousandths of a volt, a potential amplitude

that would deflect the spot of light in the most sensitive cathode ray oscillograph yet made only a few thousandths of a millimeter, whereas, to be legible, a deflection 6000 to 8000 times as high is required. Since the discovery of the thermionic amplifier,

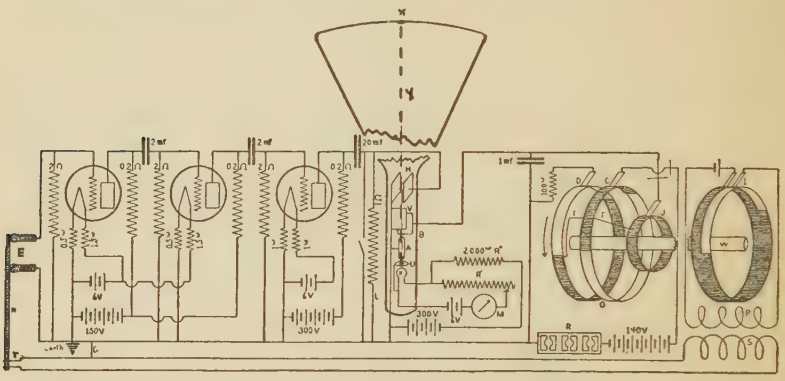


FIG. 1. Diagram of Braun tube (cathode ray oscillograph) arranged for observation of nerve action potentials. N = nerve, killed end shaded. E = non-polarizable electrodes connected through the three-stage amplifier with the horizontal plates, H, of the Braun tube, B. K = Wehnelt cathode; A = tubular anode; U = diaphragm; L = leak on horizontal plates; M = ammeter; R' = regulating resistance in filament circuit, K; R'' = resistance in anode circuit. The vertical plates, V, connect with the spreading device, O. D = discharging wheel, C = charging wheel, and J = continuous contact wheel (metallic parts shaded), all connected conductively by F; R = charging resistance to 1 mf. condenser. Y = undeflected electron stream, and X = fluorescence produced by it on screen of tube. I = rotating interrupter in primary circuit, P; S = secondary coil; T = stimulating electrodes grounded through G. 1Ω = 1 megohm. For the intertube leaks labeled 2Ω, 0.5Ω was used. More recently the "spreading" apparatus has been changed so that the condenser, *1 mf.*, is charged directly and continuously from the battery, *140 v.*, and discharged through the commutator.

however, there has existed the possibility of building nerve currents up to this required amplitude, and it was to this problem that my colleague, Dr. H. S. Gasser, who had been using an amplifier in another connection, and I first turned our attention. In the further development of this technique and in the study of

some of the phases of nerve physiology to which the new technique was applied, Dr. Gasser and I were later joined by my associate, Dr. Geo. H. Bishop. Through these studies it has been found that the required height can be given the action potential by a three-stage amplifier of the design pictured in Fig. 1.

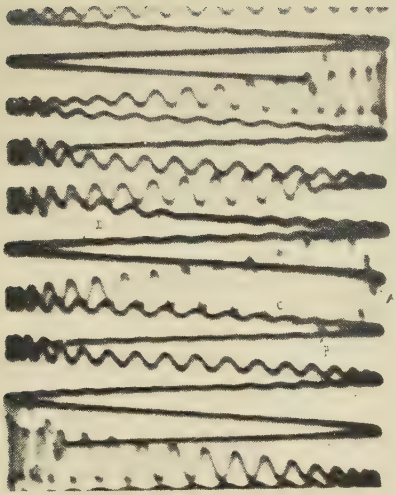
Though it is a comparatively simple matter thus to amplify nerve potentials, one has to be sure that the added amplifying device, through its reactance, does not materially deform the action waves it passes on to the oscillograph. By way of control the degree has been determined to which the system deforms waves of the same period as the nerve action waves. For this purpose records have been made of a potential oscillating at a rate of over 1000 vibrations per second, led into the oscillograph first, directly, and then, indirectly through the amplifying system but after proportional reduction in amplitude. When these two records are compared by placing them one above the other so that the points vertically over each other are synchronous, it becomes clear (fig. 3) that if there is any lag of the phases of the amplified oscillations (lower) behind those of the unamplified (upper), it is well within the limit of error. The cathode ray oscillograph, therefore, becomes available for the study of nerve and of other types of physiological action potentials.

A word is still necessary regarding the method of obtaining records. The electron stream (Y) on its way to the fluorescent screen passes between two pairs of plates set at right angles to each other (V and H, fig. 1). A potential difference between the vertical pair of plates (V) bends the stream in the horizontal plane and the spot of light consequently moves horizontally across the fluorescent screen. By means of a rotating commutator (see fig. 1) this bending, in our experiments, is repeated regularly 10 to 20 times a second, the observer thereby receiving the impression of a continuous streak of light across the face of the tube. The same commutator also times the stimulation of the nerve, the stimuli being so adjusted that during each horizontal deflection of the electron stream an action potential, after amplification, reaches the other, the horizontal, pair of plates (H).

The field thus produced between these plates bends the electron stream upwards and the action potential, being thus retraced upon the face of the tube, say 20 times a second, and always in the same position, becomes visible as a standing wave which can be photographed by the simple expedient of pressing a film to the face of the tube for a period of 2 to 3 seconds. All of the records to be thrown upon the screen have been thus obtained.

It is, of course, necessary, for purposes of analysis, to be able to measure the records in terms of time. Now the length of record subtending a unit of time naturally depends upon the speed with which the spot of light moves horizontally across the screen, and this movement is determined, as has been said, by the potential that is applied to the vertical pair of plates. The device we use for controlling and varying this timing potential consists of a fixed condenser charging, in effect, from zero to a fixed level through a resistance. Since the rate with which the condenser fills depends upon this resistance, the rate with which the spot of light moves can readily be controlled; and this rate can be determined by a comparatively simple computation. It should be recalled, though, that the potential of a charging condenser rises logarithmically, that is to say, more and more slowly as the condenser fills; therefore the distance the spot moves in successive units of time also diminishes logarithmically. This may be seen in any of the figures in which the successive units of time, usually thousandths of seconds, or sigmas (σ), ascertained mathematically, are marked upon the records.

The rate of movement of the spot of light horizontally corresponds, so to speak, to the rate of movement of a kymograph, to illustrate by means of an instrument with which many of you are familiar. Now a kymograph driven at the rate of 1 m.p.s. is, as you may know, moving close to its practicable limit. Very much higher speeds are attainable through the oscillograph. In figure 3, for instance, the mean rate of the spot of light during the first half sigma was about 50 m.p.s., i.e. to say 50 times the kymograph rate just mentioned, and this is by no means the practicable limit. The high speed attainable with the cathode ray oscillograph constitutes another of its advantages over the



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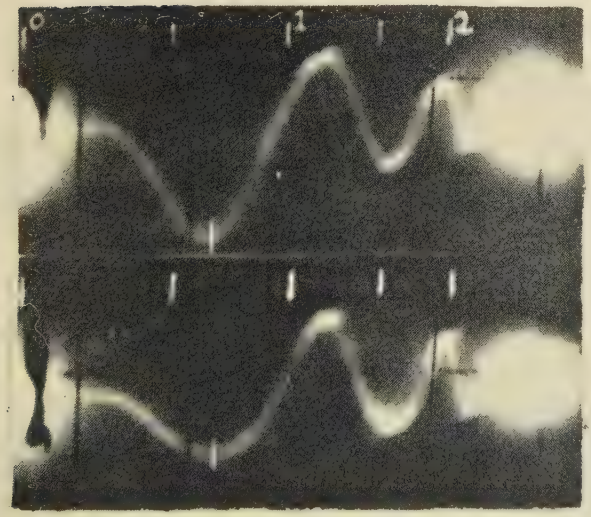

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FIG. 2. Oscillations at the rate of 200,000,000 per second recorded by the cathode ray oscillograph. From Dufour.

FIG. 3. Records of decremental sine waves through the amplifier (lower) and onto the oscillograph direct. The time marks are $0.5\,\sigma$ apart. $\times \frac{3}{5}$.

methods heretofore employed in physiology; and these speeds, it may be added, are essential when dealing with phenomena, such as action potentials, some of whose phases need to be measured to within a hundred thousandth of a second.

The nerves employed in these experiments for the most part have been removed from the body. Omitting reference to certain technical precautions which must always be observed in experi-

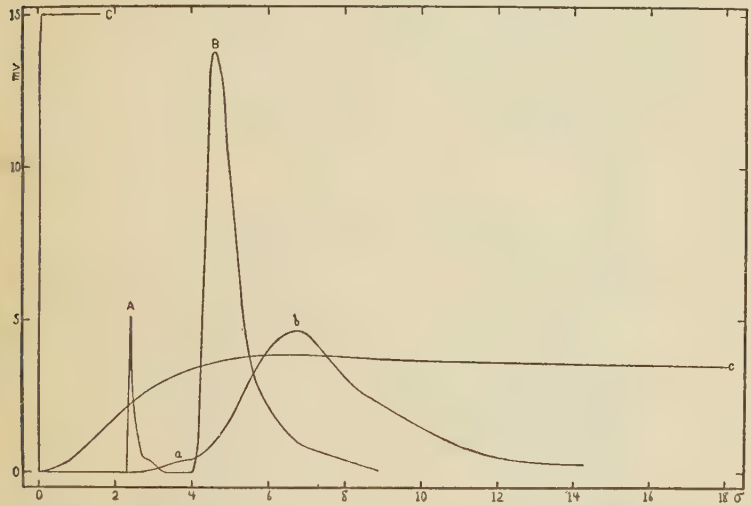


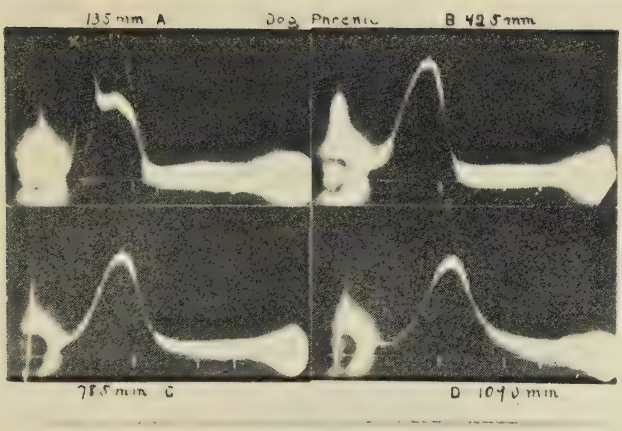
FIG. 4. Action potentials of the bullfrog sciatic, as recorded by the Braun tube and string galvanometer, plotted in rectangular linear coördinates. *A*, *B*, *C*, Braun tube records; *a*, *b*, *c*, string galvanometer records. *A*, *a*, shock; *B*, *b*, action potential; *C*, calibration with a constant current of 15 mv.; *c*, with one of 3.75 mv.

ments on nerve, and with which most of you are familiar, I may merely point out to you the position occupied by the nerve, *N*, in our scheme (fig. 1). *T* labels the electrodes through which the nerve is artificially stimulated, and *E*, the leads through which the resulting nerve action potentials are picked up, after conduction along the nerve, and carried into the recording apparatus.

With the methods heretofore used such nerve preparations have given records without distinguishing features. One searches

the literature in vain for statements even suggesting the possibility of characteristic action potentials. The configuration of the action potentials obtained with the cathode ray oscillograph, on the other hand, depends, as will be seen, upon the nerve employed and upon the distance the action potential has moved along the nerve. The reason for the differences in the results obtained by the older and by the new methods is seen in figure 4. The time in this figure is indicated on the base line in thousandths of seconds. A constant potential instantly applied to the string galvanometer, one of our best recording instruments, produces a deflection (*c*) which requires about 0.005 seconds to reach its maximum. An instantaneous potential applied to the cathode ray oscillograph through the amplifier reaches its full height (*C*) in a few hundred thousandths of a second. *A* and *B* are respectively the escape of the stimulating induction shock and the resulting action potential wave of a nerve as recorded with the cathode ray oscillograph. *a* and *b* are identically the same potentials taken from the same preparation but by the string galvanometer. The quicker oscillograph records as entities the escape of the stimulating induction shock and the action potential; whereas these features in the record given by the string galvanometer are merged and are considerably later than in the oscillograph record. Such a lag of the string behind the events to be recorded, obviously is bound to result in inaccurate timing and in the masking of all of the finer details of processes transpiring as quickly as those of nerve.

So much for method. By way of introduction to the results the method has yielded we may first describe the action potentials obtained from the two nerves most commonly employed in our investigations, the phrenic nerve of the dog and the sciatic nerve of the bullfrog. The phrenic nerve yields an action potential which always is simple, but which changes its form somewhat as it moves away from the point whence it is started by the induction shock. Figure 5 shows this wave at points on the nerve distant from the place stimulated, 13.5, 42.5, 78.5 and 109 mm. in *A*, *B*, *C* and *D*, respectively. The deflection in *A* was so high that, in order to bring it within the limits of the screen, it was



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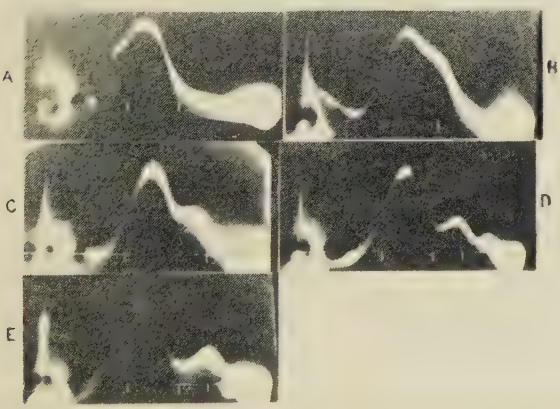

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FIG. 5. Contact prints of the action potential in the phrenic nerve of the dog at the four conducting distances indicated. Temperature = 37.5°C. The marks extending below the base line indicate successive σ. $\times \frac{1}{2}$, approximately.

FIG. 6. Contact prints of the action potential in the sciatic nerve of the bullfrog. Conducting distances in millimeters: A = 12.0, B = 31.0, C = 46.5, D = 62.5, E = 82.0. Temperature = 26°C. A, shunted somewhat. $\times \frac{1}{3}$, approximately.

necessary to shunt a part of the potential; the dotted curve shows the action wave redrawn in its unshunted height, and comparable, therefore, with the other records of the series. The peak, *X*, is the escape of the stimulating induction shock which here is applied so close to the lead from the nerve that it spreads conspicuously into the record. Disregarding this escape, it is clear that the potential wave diminishes in amplitude and broadens as it moves along the nerve.

Figure 6 shows the action potential in the sciatic nerve of the bullfrog picked up at five distances from the point stimulated. The picture differs strikingly from that given by the phrenic. Disregarding again the deformation (on the ascending limb of *A*) due to the escape of the stimulating induction shock, it can be seen that though the action potential that has traveled but a short distance, 12 mm. (*A*), apparently is simple, at a point 31 mm. down the nerve (*B*) the descending limb of the record evidently is becoming complicated. The nature of this complication becomes clearer as the action potential moves further along the nerve. At 46 mm. of conduction (*C*) two waves have begun to separate out from the first, and the distance between the three waves obviously increases with further progress of the action potential along the nerve. These crests on the action potential, of which in the sciatic there usually are three, sometimes four, we have designated noncommittally as *alpha*, *beta*, *gamma* and *delta*. Furthermore, it can be seen in these records that, like the simple action potential wave of the phrenic, each of the waves of the sciatic action potential diminishes in height and broadens as it moves along the nerve.

When one transfers these records from their logarithmic system of coördinates, in which the successive time intervals diminish from left to right, to the linear system (fig. 7) in which the time intervals are equal, and then arranges the curves thus obtained one above the other by placing their starts on the same vertical, and in addition, spaces them vertically by distances which are proportional to the respective distances of conduction, it becomes evident that the crest times of corresponding waves fall upon straight (the dotted) lines. This result signifies, if the front of

the first wave is travelling at a uniform rate, and it can readily be proved that this is the case, that the other waves also are moving along the nerve at uniform but slower rates; and if the rate with which the start of the action potential travels be directly determined it becomes possible from such records as these to calculate the propagation rates of all of the waves. In the case of the sciatic nerve of the bullfrog at room temperature these rates have usually been in the vicinity of 42, 25 and 17 m.p.s. for *alpha*, *beta* and *gamma* respectively. *Delta* is so small that it has not been possible to determine its rate accurately. It would appear, however, that it travels at the rate of about 12 m.p.s.

Experiment has demonstrated that these waves develop because of group differences in the rates with which the constituent fibers of the sciatic nerve conduct their action potentials. That this is the case is indicated in the first place by the effect stimulation strength has upon the form of the action potential wave. If one leads from the nerve at a distance from the site of stimulation, where the waves have so separated one from the other that they can be readily recognized, and records the effect upon the configuration of the action potential of gradually increasing the strength of the stimulus, it is found that at the threshold the action potential consists merely of a small *alpha* wave. Upon gradually increasing the strength of the stimulus this *alpha* grows in height and at about the time it reaches its maximum a minute *beta* wave appears. This then grows as did *alpha*, and the *gamma* wave appears, and so on. Figure 8 shows, among other things, the action potential in the frog's sciatic at two stages of such an experiment, namely, (*B*) as produced by a stimulus just about maximal for *alpha*, but below the threshold of *beta*, and again (*A*) after increasing the stimulus to the point where *beta* becomes maximal. Such a result indicates that the fastest travelling wave, namely, *alpha*, comprises the action potentials in the most irritable of the fibers, that the *beta* wave is made up of the action potentials in somewhat less irritable fibers, and so on.

It is, indeed, possible to prove beyond peradventure that separate groups of fibers contribute their potentials to the *alpha* and to the *beta* waves; and, it is justifiable to conclude, therefore, that

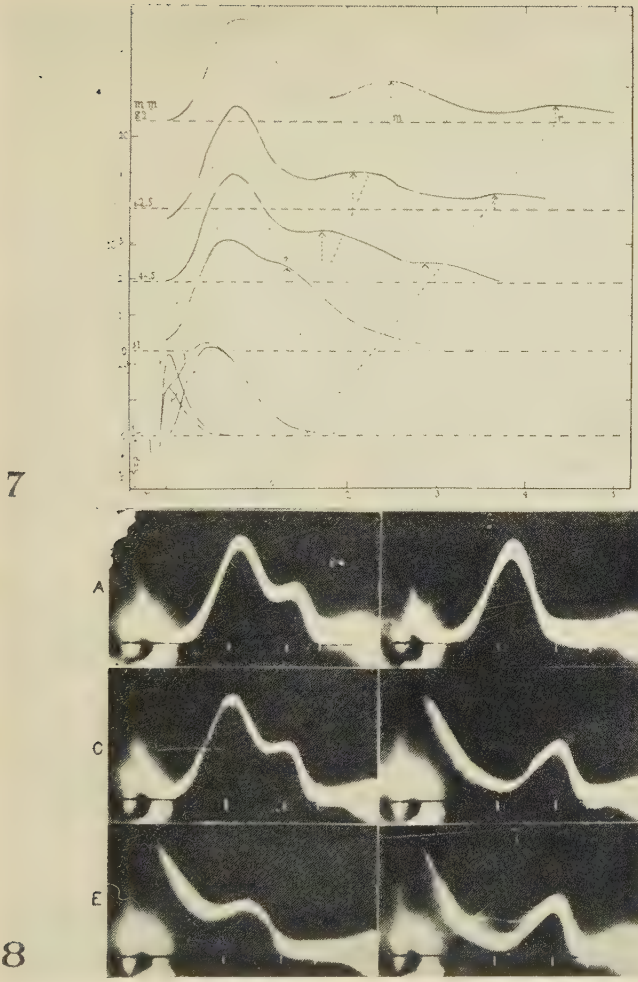

FIG. 7. The records of figure 6 transposed onto rectangular, linear coördinates. Abscissae give the time in σ. Ordinates: *Sep* = conducted distance, with zero at the *X* axis; *Ht* = amplitude of the action potential in millimeters, the zeros being the base lines of corresponding records.

FIG. 8. Records of the action potential in the bullfrog's sciatic nerve illustrating the isolation of the *beta* process by stimulating first with an induction shock that is maximal for *alpha*, but subminimal for *beta*, and then, after an interval short of the absolute refractory phase, with a stimulus that is maximal for both. *A* = first action potential alone, stimulus strong; *B* = first action potential alone, stimulus below the threshold of *beta* and *gamma*; *C* = second action potential alone, stimulus strong; *D* = *C* following *B* (carried forward) by 0.85σ; *E* = *A* carried forward as while making *D*; *F* = same as *D* but with unsteady first stimulus. The lines extending below the base line indicate successive σ. $\times \frac{1}{2}$, approximately.

this applies also to the *gamma* and *delta* waves. In view of the fundamental importance of this fact the experimental evidence proving it will be presented in some detail. One line of attack uses, to attain the end in view, the phenomenon of refractoriness displayed by nerve and the difference in the irritability of the *alpha* and *beta* fibers. First there is passed through the nerve an induction shock that is strong enough to stimulate all of its fibers, or, as in this case, all of its *alpha* and *beta* fibers. It produces the action potential, *A*, in figure 8. The shock is then carefully weakened to the point where it stimulates the *alpha* fibers but none of the *beta* fibers (*B*, fig. 8). And finally the nerve is stimulated with this last shock and, almost immediately thereafter, while the *alpha* fibers still are refractory as a result of this response, with the shock that called forth both the *alpha* and the *beta* waves. The figure shows (*D*) that the latter elicits an action potential consisting solely of the *beta* wave. Obviously *alpha* and *beta* are produced each by its own set of fibers.

A second method, proving even more conclusively that the *alpha* and *beta* elevations are produced by action potentials travelling in different groups of fibers, is pictured in figure 9. The sciatic nerve of the bullfrog is placed upon two pairs of leads, *A*—*B* and *E*—*F*, arranged so that the monophasic action potential can be led off from either end of the nerve. Arrangements also are made to stimulate the nerve with induction shocks at two widely separated points, *C* and *D*, between these leads into the recording mechanism. The first step in the experiment consists in finding by trial the strength of stimulation, applied to the nerve at *C*, that elicits an action potential made up of both the *alpha* (α) and the *beta* (β) waves. Its record at *E* is seen in *A*. The second step consists in finding a stimulus applied at *D* that produces only a just maximal *alpha* potential wave. Its record at *E* is seen as α' in *B*. The *beta* wave (β) in *B* will be considered in a moment. Now action potentials travel along nerve in both directions from the point stimulated. Therefore, if, as the third step in the experiment, these two selected stimuli be applied simultaneously at *C* and *D*, the lone *alpha* wave travelling from *D* toward *C* will meet the *alpha* and *beta* combination travelling from

C toward *E*. If the *alphas* travelling toward each other are in the same fibers they will mutually block each other and of the α and β waves travelling from *C* to *E* only the β wave will continue to *E*. Examination of record *B* shows that though the *alpha* wave that records in *A* has entirely disappeared, the *beta* wave (β) is present and is altered neither in form nor in position.

The waves then are formed of action potentials in different sets of nerve fibers. In the action potential that is started by stimulation of a point on a nerve, waves become evident because the action potentials in the several groups of fibers travel at different speeds, slower in the *beta* group of fibers, for instance, than in the *alpha* group, the *alpha* wave, therefore, gradually gaining on *beta* until, if the distance of conduction be great enough, the two crests become quite clear of each other.

The experiments we have presented show that the fibers composing the sciatic nerve can be divided into three or four groups on the basis of their physiological properties, the fibers contributing to the *alpha* wave, for example, being the most irritable and the most rapidly conducting. These two properties of a fiber evidently are related one to the other, but whether they vary in exactly the same proportion from fiber to fiber has not been definitely ascertained. However this may be, we probably have in irritability differences a convenient if not an accurate means of classifying fibers according to the physiological properties just mentioned. Now the manner in which the action potential

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Fig. 9. Diagram (above) indicating the method, and records (below) of an experiment proving that the *alpha* and *beta* waves travel in different fibers.

A = action potential made up of both *alpha* (α) and *beta* (β) travelling from *C* to *E*.

B = the same action potential modified as a result of passing it through a maximum *alpha* process (α') going in the opposite direction. Only the *beta* process of the former remains.

C = same as *B* except that the *alpha* process (α') in this case is submaximal. Now, in addition to the *beta* process, some of the *alpha* process remains; the latter is reduced in height as compared with α in *A*. $\times \frac{3}{4}$, approximately.

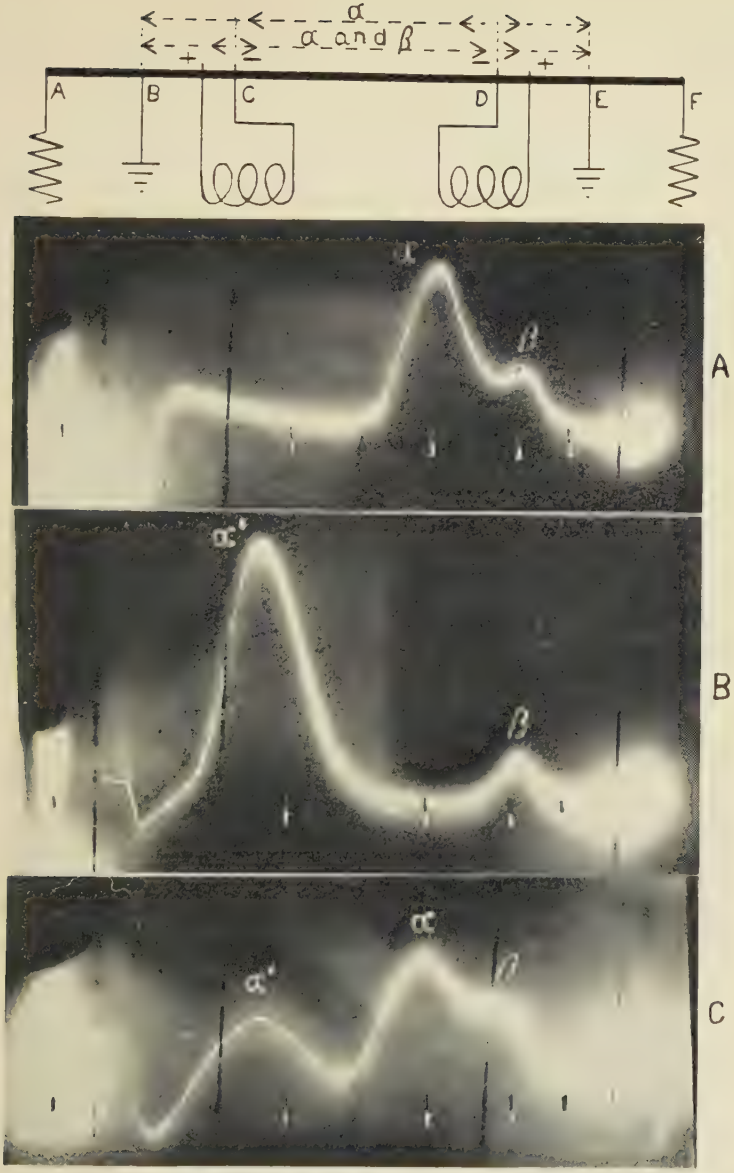

FIG. 9.

grows as the strength of a stimulating current is increased indicates that within each group, *alpha*, *beta*, *gamma*, etc., the fibers have a wide range of irritability there being usually, as a matter of fact, no gap between the least irritable fiber of one group and the most irritable of the next. Presumably, therefore, the transition of the physiological properties of the nerve fibers from those of one group to those of another is gradual, rather than sharp.

Furthermore, if it be true, as this test seems to indicate, that the individual fibers contributing to an action potential wave conduct at rates distributed between that of the fastest and that of the slowest of the fibers contributing to that wave, it must follow that this circumstance, and not so-called conduction with a decrement, accounts for the lowering and the broadening of the waves as they move along the nerve. What would transpire in a nerve so constituted is visualized in figure 10. This figure portrays an *alpha* action wave composed of potential waves travelling at slightly different, though regularly distributed, rates in the individual fibers. For the sake of simplicity the diagram is made to depict the action waves in only 5 (*1*, *2*, *3*, *4*, *5*) of the many fibers ordinarily participating. Since they are all travelling at different rates the waves lag one behind the other as they move along the nerve from the point stimulated like so many men running from scratch at different speeds. The action potential they produce at any given distance of conduction obviously is the sum at that point of the five fiber or *axon* potentials, as we shall now designate them. At zero distance, that is, at the point where the nerve is artificially stimulated (at scratch so to speak), all of the five waves exactly superpose and sum to form the wave *0*; but as they pass, say, the 8 cm. post the fastest wave is in the lead and the remaining waves are strung one behind the other as shown. At this point the potentials sum to form the (lower) wave *8*. Obviously these premises would satisfactorily account for lowering and broadening of the action potential during conduction; as a matter of fact, all of the experimental data support this conception. It will suffice, however, to present only one of the several expedients to which we have resorted in putting this hypothesis to the test.

Reference has been made to the fact that a fiber that has responded to a stimulus will not respond to another until a certain interval of time has elapsed. In order that we may have a convenient measure, let us say, even if it may not be quite true, that this refractory period lasts the duration of the axon action potential. It is then obvious that despite the steady increase in the duration of the composite potential wave as it moves along the nerve, the refractory period always will terminate this constant distance from the beginning of the action potential. In other words, the end of the action potential should fall further and further behind the termination of the absolute refractory period. This, experiment proves to be the case.

Furthermore it must follow, if the action wave is thus compounded, that, contrary to the well known fact that when two successive stimuli are applied at one and the same point the nerve becomes refractory immediately upon responding to the first, i.e., at the instant the action potential begins to develop locally, the nerve at a distance from the point artificially stimulated does not become wholly refractory the instant it is reached by the travelling action potential wave. Thus, turning again to the diagram (fig. 10), a second stimulus applied at zero distance of conduction, say, 0.0002 of a second after the first, would find all of the fibers active and consequently refractory; the stimulus would have no effect whatever upon the form of the potential wave. But if the two stimulators had 8 cm. of the nerve between them and if the second stimulus were delivered, again, 0.0002 of a second after the beginning of the action potential had reached the nerve at the position of the second stimulator, it is obvious that it would find only the fastest fibers (*1* and *2*, fig. 10, 8 cm.) active and therefore

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Fig. 10. Diagrammatic representation of an action potential wave based upon the assumption that it is composed of the sum of similar axon action waves conducted at different, but regularly distributed, rates. The diagrams picture five axon waves (*1*, *2*, *3*, *4* and *5*) travelling at the rates of 42, 39, 36, 33 and 30 m.p.s., respectively, and their sum (divided by 2 in order to obtain a convenient height) at distances from the site of stimulation ranging from 0 to 10 cm. as indicated on the vertical axis. Abscissae, time in σ.

FIG. 10.

refractory, whereas the slower fibers, *3*, *4* and *5*, not yet having become active, would still be irritable and would respond. The action potentials of these three fibers all would develop under the stimulus at that instant, and the form of the recorded action potential would be altered (upper wave at *8 cm.*) through their summation at that point. Figure 11 shows that this is the result actually obtained experimentally. Record *B* is an action potential wave 8 cm. from the point it originated; and record *C* shows the result of applying a second stimulus at a point 8 cm. distant from the first stimulus and 0.0002 seconds after this action potential had begun to develop there. The alteration it produces in the configuration of the wave is exactly that demanded by the premises (see 8 cm., upper wave, fig. 10).

Since an action potential wave started by artificial stimulation changes its form as it moves along the nerve, it is obvious that the efforts heretofore made to establish the form and the time constants of the action current and to correlate these data with the fundamental mechanism of nerve activity have been largely misdirected. Investigators were dealing not with constants but with values varying with the length of nerve intervening between the stimulating electrode and the lead into the recording instrument. Now that we know that the building stones of the compound action potential are the action potentials of the individual fibers, the *axon potentials*, in other words, our efforts obviously must be directed toward acquiring knowledge regarding the characteristics of the individual axon waves and of the fibers in which they develop.

There are several procedures that may be followed in order to obtain information relative to the time constants of the axon action wave. One is illustrated in figure 10: the duration may be determined of the action potential at any two or more distances of conduction; from these values the duration of the wave at zero distance of conduction may be derived by the method of extrapolation. Another method is to lead into the recording mechanism the action potential developing at the point where the nerve receives the artificial stimulus, for here all of the axon potentials are still in phase. This, however, is not a simple procedure tech-

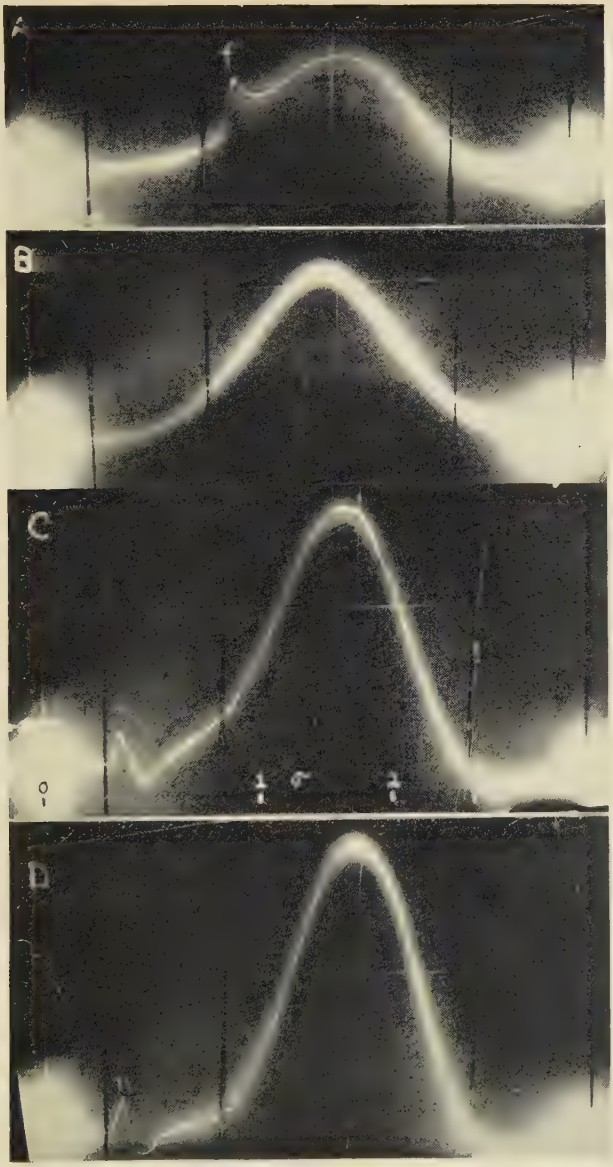


FIG. 11. Records from an experiment demonstrating the absence of a refractory phase under the earlier stages of a conducted *alpha* action wave. Bullfrog's sciatic. The vertical rulings are on the face of the tube and indicate comparable time points in all of the records excepting *A*. $\times \frac{2}{3}$.

nically, for the potential of the stimulating current then escapes into the recording mechanism and, as has been seen, tends to cover the action potential that develops in the nerve. Despite this difficulty we have, by balancing out the escape, and by using a nerve with a thin sheath, succeeded in obtaining satisfactory records of the action potential at or very close to the site of its origin. The two methods agree in showing that the time constants of the axon action potentials in any given nerve, at least, are all very nearly alike. In the sciatic nerve of the bullfrog, for example, all axon waves under the conditions of our experiments reach their crests in about 0.0004 seconds and terminate in about 0.0014 seconds; and in the phrenic of the dog, to cite results obtained in a warm-blooded nerve, in less than 0.003 and 0.007 seconds, respectively.

The evidence that has been presented leaves no room for doubting, then, that the individual fibers composing a nerve conduct at different rates and that the conduction rates of the component fibers of a given nerve are such as to produce action potentials with patterns that are more or less characteristic, and we may now turn our attention to certain questions that these findings suggest, such, for example, as,—is it possible to recognize the fibers that conduct at the different rates; is there any law governing the rate with which a fiber conducts; and again, is there any relation between the groups of fibers in a mixed nerve and the functions they mediate?

In our efforts to find the answers to these questions we have, along with other procedures, examined various nerves in order to ascertain whether there is any relation between their fiber histology and the configuration of their action potentials. It has long been known that different nerves may differ in their morphology and, especially, in respect to the diameters of the fibers composing them. And even a superficial comparison of a conducted action potential with the histology of the nerve that produced it was sufficient to indicate that a more careful investigation of this relationship might prove interesting. Microphotographs were, therefore, made of nerves magnified 1000 diameters or more and the myelinated fibers in these enlargements were measured so as to obtain their mean outside diameters. Then, in order to visual-

ize the data thus obtained, they were plotted as shown in figure 12 (lower graph). Here each dot represents a fiber measured. The values on the horizontal axis of the graph give the diameters of

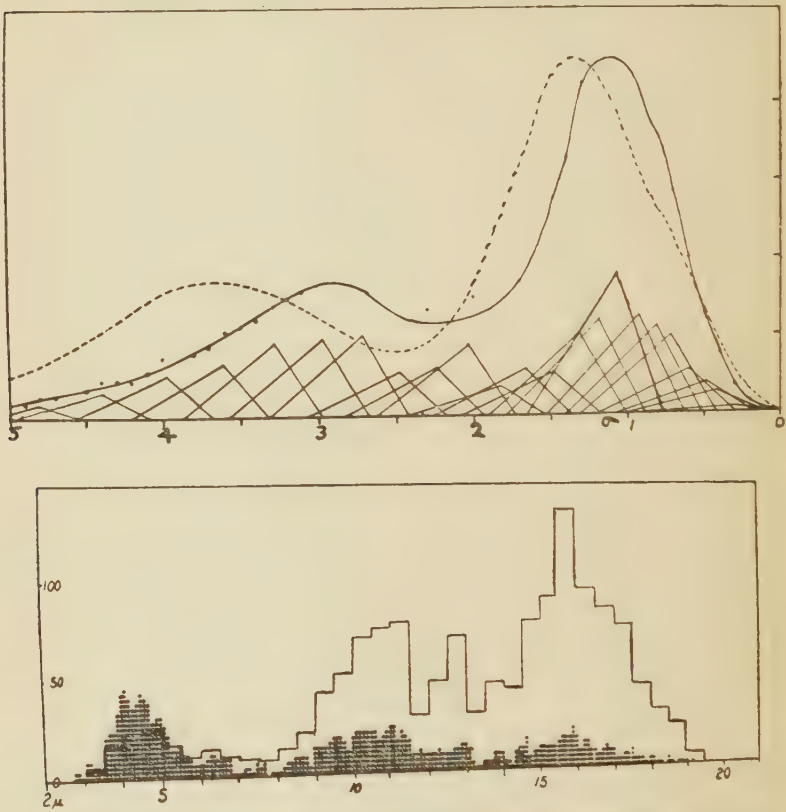


Fig. 12. Lower. Distribution curve of all the fibers (377) in the peroneal nerve of the bullfrog.

Upper. Solid curve: Reconstruction of the peroneal action potential at 146 mm. Dotted curve: Corresponding recorded action potential.

the fibers in thousandths of a millimeter, i.e., in microns or μ. The number of dots over a given figure represents the number of fibers of that size. The dots on the vertical line marked 15, for instance, represent the fibers measuring 15 microns in diameter,

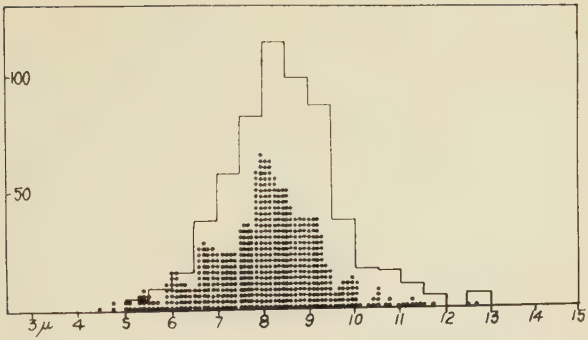
on the vertical line marked 10, the 10 micron fibers, and so on. This graph gives the results obtained through measurement of all of the myelinated fibers, 377, in a mixed nerve, the peroneal of the bullfrog. It exhibits three definite crests made by fibers ranging in size between about 19 and 13, 13 and 7 or 8, and 7 or 8 and 2 microns, respectively. The action potential in this nerve, it will be recalled, ordinarily exhibits three, sometimes four, crests. It is conceivable that the unmyelinated fibers, which we have not measured, might form a fourth crest. Figure 13 pictures the results obtained through a similar analysis of a phrenic nerve of the dog. The diameters of the phrenic fibers, it is seen, cover a fairly wide range, yet they group themselves so as to form but a single crest. The action potential of this nerve it will be recalled likewise presents but one crest.

Such correspondences between fiber pattern and action potential pattern indicate that there is some relation between fiber size and axon conduction rate. In the effort to ascertain what this relation might be we have proceeded along the following lines. It is clear that one could reconstruct the action potential of any nerve at any given distance of conduction if the following data were available: (1) the configuration of each of the axon potential waves, (2) the magnitude of the deflection that each produces in the oscillograph and (3) the rate with which each fiber conducts. (1) Now, to consider the first requisite, it has already been stated that the axon potentials in any given nerve all are approximately alike in configuration and that this configuration can be ascertained by recording the action potential at the point in the nerve where it is started by artificial stimulation. In order to lessen the labor of construction, the axon potentials have been given the form of triangles, a departure from fact that is not sufficiently great to materially alter the results derived; but the ascending and descending limbs of these triangles have been given durations agreeing with the lowest values ascertained by direct measurement. (2) With regard to the magnitude of the deflection, there are reasons for believing that the inherent potentials also are alike in all fibers. On this basis it may be inferred that the effect produced by each potential upon the recording instrument will be

inversely proportional to the electrical conductivity of the fiber, or, in other words, directly proportional to the cross area of the fiber. (3) And finally, with regard to the conduction rate, to consider the third requisite, it is possible to determine directly only the rate at which the fastest axon wave moves, this being the rate of movement of the front of the action potential as a whole. The rates in the other fibers can be ascertained only by making pertinent assumptions and then ascertaining whether on their basis the configurations of specific nerve action potentials are reproducible. The assumption that has given the best results is that the rate with which the fibers conduct their action potentials is proportional to their diameters.

A result based upon such an assumption is illustrated in figure 12 (upper graph). Here, in order to reduce still further the labor of the reconstruction, we have treated as one fiber all of the fibers contained within a range of 0.5 microns. The relative cross areas of the fibers, thus reduced in number, or, in other words, the relative amplitudes of their action potentials, are indicated by the height of the treads of the solid, stair-like curve (lower graph). The triangular action waves, with amplitudes thus derived, are then placed one behind the other in the upper graph, spaced from zero by the times they would lag on account of their assumed slower conduction rates while running a distance of 146 mm. The combined effect these axon waves would produce upon the oscillograph is given by their sum, which is represented by the solid line curve. Now, the very nerve of which this is the histological analysis, with the oscillograph gave at the same distance of conduction, that is 146 mm., the action potential shown in this figure in the form of the dotted curve. A similar reconstruction of the phrenic action potential, made to bring out the form it would have at a distance of 80 mm. from the site of stimulation, is shown in figure 14, and alongside it the record obtained from the same nerve at the same distance of conduction. Both have but one crest, and indeed closely resemble each other in every respect.

The close agreement between these reconstructed curves and the actual action potentials justifies the conclusion that, to a first approximation, the conduction rate of the impulse in nerve fibers



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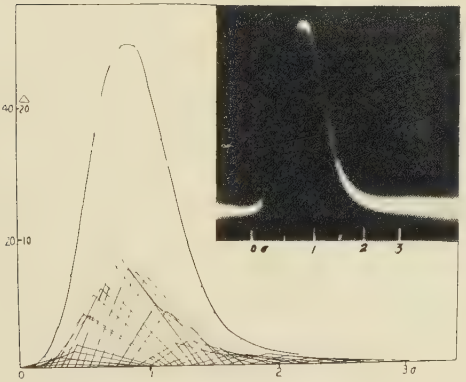

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Fig. 13. Distribution curve of fibers in the phrenic nerve of the dog. Partial analysis; 637 fibers or a little over one-third.

Fig. 14. Reconstruction of motor root action potential at 48 mm. of conduction. The triangles are plotted on twice the scale of the summation line.

Inset: Action potential of the motor root as recorded 48 mm. from the stimulus.

is proportional to their diameters. This being the case it follows that from the fiber size pattern of a nerve one can ascertain the fibers that contribute to each of the features of the conducted action potential. Thus, in the sciatic or peroneal nerve the *alpha* crest is formed by the group of large fibers, *beta* by the group of intermediate fibers and *gamma* by the group of small fibers, all, it should be remembered, being myelinated.

We now come to the question, is there any relation between this evident grouping of fibers in respect to size and the functions mediated by the groups. Though some progress has been made in the quest of this information, much still remains to be done. Brief reference may, however, be made to some of the more significant and relevant of the data thus far accumulated. It is possible, in the bullfrog, to remove without injury the sciatic nerve together with any one of its several pairs of spinal roots. Such a preparation may be arranged as shown in figure 15. Stimulation at S_1 starts an action potential in fibers contributed to the nerve by the two spinal roots remaining attached. The action potential thus started, when led from the sensory root, resembles very closely that obtained from the sciatic nerve itself. It is made up of *alpha*, *beta* and *gamma* waves as may be seen in figure 16 (lower record). Under exactly the same circumstances the action potential that reaches the motor root (upper record) is simple in form and in most of its characteristics resembles an *alpha* wave. The motor wave and the first, that is to say, the fastest, wave in the sensory action potential travel almost exactly at the same rate, and, therefore, in the sciatic action potential, are indistinguishably merged to form the *alpha* elevation. The *alpha* wave in the sciatic nerve, therefore, is formed by both motor and sensory fibers, whereas the *beta* and *gamma* elevations are produced by fibers reaching the nerve by way of the sensory roots only. The source of the potential that produces the indistinct and inconstant *delta* elevation is not definitely known, though, as has been said, we are inclined to attribute it to the action potentials travelling in unmyelinated fibers which are certainly efferent and probably afferent, also.

To take another case, the femoral nerve of the dog breaks up,

in the thigh, into the saphenous nerve and nerves going to voluntary muscles, the former containing fibers mediating skin functions only. If one makes a preparation of this nerve comparable to the sciatic-root preparation (see fig. 15) but consisting, instead, of femoral nerve with one of its muscle branches and the saphenous branch, and if one leads from each of the branches separately the action potential started by stimulation of the common trunk, it is found that the muscle nerve action potential has but one crest, the saphenous nerve action potential what may be regarded as three crests; furthermore, it is found that the front of the action potential in the muscle nerve travels faster than in the saphenous. The explanation of these differences has recently been found through the construction of the graphs of fiber size distribution seen in figure 17. The fibers of the muscle branch, lower graph,

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FIG. 15. Diagram of the sciatic nerve of the bullfrog with one of its pairs of roots (*VIII*) arranged on the leads and stimulators most commonly employed in experiments tracing the action wave from nerve trunk into roots. *g* = ganglion; *d* = dorsal branch; *r* = branch communicating with sympathetic chain; *VII* = 7th spinal nerve; *IX* = 9th spinal nerve; *f* = femoral nerve; *m* = first large muscular branch; *t* = tibial nerve; *p* = peroneal nerve. S_1 and S_2 = stimulators; E_m, E_c, E_s and E_m = ground leads on nerve trunk, at crotch of roots, on sensory root and on motor root, respectively; only one of these leads is in contact with the nerve at a time. G_s and G_m = grid leads from ends of sensory and motor roots; through the switch, *k*, these can be connected with the recording mechanism either separately or together.

FIG. 16. Records showing the action potential in the ventral root (upper) and in the dorsal root (*VIII*) (lower) of a bullfrog's sciatic preparation. The stimulation strength was the same, and maximal, for both records. The upper record is simple; the lower gives evidence of *beta* and *gamma* in addition to *pre-alpha* and *alpha*. The marks on the lower base line indicate successive σ for both records. × ⅔, approximately.

FIG. 17. Fiber distribution curves of two branches of the femoral nerve of the dog. *M* = a muscle branch (372 fibers, or about ⅛ of area measured); *S* = saphenous nerve (390 fibers measured, and 330 estimated, together representing about ⅛ the area). Dots represent fibers whose diameters were actually measured, circles those estimated.

FIG. 18. Action potential in a skin nerve of the bullfrog. The deflection prior to *X* is the escape of the induction shock. The marks indicate successive σ. Natural size.

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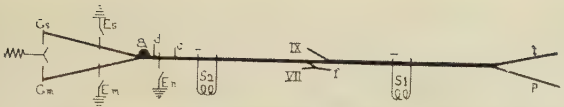

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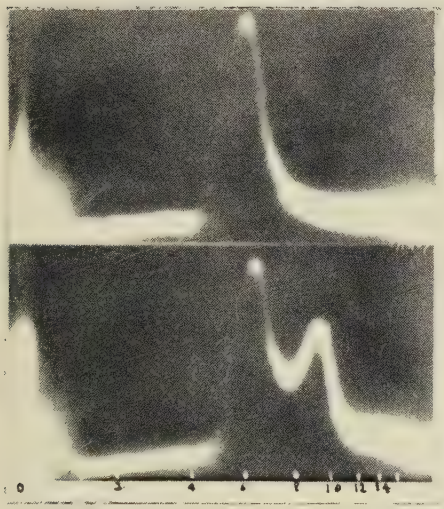

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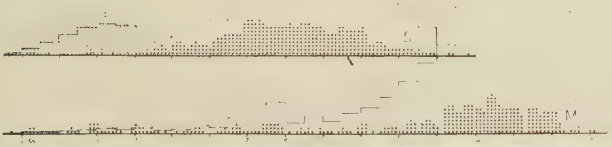

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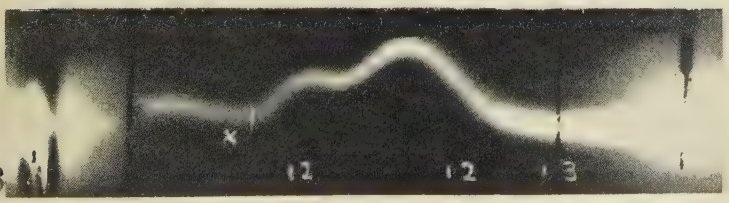

are mainly large and group themselves so as to form but one main crest. The medullated fibers of the saphenous nerve, upper graph, fall into two main groups. It can readily be seen that by combining these two graphs a picture is obtained which resembles very closely that derived through analysis of the medullated fibers in a mixed nerve, the peroneal of the bullfrog for example (see fig. 12). Evidently the saphenous nerve does not contain the group of large fibers that form the *alpha* wave of the action potential in the sensory root. These must, therefore, be in the muscle branches, and the graph shows that they do contain all of the larger fibers. The large, or *alpha*, sensory fibers thus prove to be concerned with so-called muscle sense; and it follows that the first wave in the saphenous in reality is homologous with the *beta* wave of the mixed action potential. The action potential in the muscle branch resembles closely that yielded by the phrenic nerve; and it is now easy to understand why there should be this resemblance: it is unlikely that in either of these nerves there are many sense fibers of the kind concerned with the modalities of the skin senses. It may be worth while emphasizing here the fact that both on the motor and the sensory sides the muscles of the limbs are supplied with the quickest of the nerve fibers. It must obviously be to the advantage of the organism to possess, on both the efferent and afferent sides, quick intercommunication between the central nervous system and the muscles employed in defense and in the securing of food.

To cite just one more instance,—in the bullfrog there are skin nerves crossing the dorsal lymph sac, which are quite free over a length of 2 to 3 cm. and therefore are readily obtainable for examination. Though these nerves are short they nevertheless yield action potentials that suffice for present purposes. These invariably exhibit two waves (fig. 18). The conduction rate of the faster wave is considerably slower than that of the leading wave in the sciatic nerve of the same animal; indeed, it moves a bit faster than the notch between the *alpha* and *beta* waves in the sciatic. Microscopical examination shows the largest fiber in this skin nerve to be considerably smaller than the largest fiber in the sciatic. Undoubtedly, therefore, as in the skin nerve of the

mammal, the skin nerve of the frog is made up of the *beta* and *gamma* fiber groups (and presumably *delta*, also), the *alpha*, or muscle sense group, being absent. There is, however, one difference: in the mammalian nerve the first, here, the *beta*, wave is the higher, whereas in the amphibian skin nerve it is the second or *gamma* group of fibers that is best developed. What may be the significance of this fact it is at present quite impossible to say.

Such examples as these serve to indicate the stage to which experiments of this type have carried us in the effort to assign functions to the fibers that produce the main waves of the compound action potential. They show that in the sciatic nerve, for example, the *alpha* elevation is produced by motor fibers and by muscle sense fibers. They show, furthermore, that the *beta* and the *gamma* elevations, wholly, and the *delta* elevation, in part, presumably, are produced by sensory impulses,—and that is all. Efforts, however, are being made to acquire further information through other methods of approach, but the new experiments have not proceeded far enough to warrant any very definite deductions at this time. In order, however, to illustrate one of the possible methods of attack a word may be said regarding one of the more recent findings. If, in the anaesthetized dog, one cuts the saphenous nerve and notes, while stimulating, the central end of the nerve with induction shocks gradually increasing in strength, the relation of the development of certain reflexes to the growth of the action potential waves, one finds that those reflexes, namely, a marked rise in arterial pressure and a marked hyperpnoea, that are regarded as indicative of stimulation of pain fibers, usually begin to appear as the stimulus becomes strong enough to bring out the *delta* wave. It seems probable therefore that a part, at least, of the *delta* fibers is concerned with pain. On this basis the *beta* and the *gamma* groups of fibers would remain for the mediation of the touch and of the temperature senses.

In conclusion it ought to be made clear that in speaking as we do of fiber groups it is not our intention to give the impression that the fibers concerned with a given function are limited sharply to the confines of the corresponding fiber mound. As a matter of fact it is clearly evident that this is not the case; the adjacent fiber

groups very definitely overlap somewhat. Nevertheless the distribution of the fibers is such as to bring about the massing around certain points that gives to the action potential features which, with increasing knowledge, should prove helpful in the efforts to establish the relations between nerve fiber and function, and between reflex responses and the modalities of the senses eliciting them.

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HEALTH AND ACTIVITY[1]

de motu corporis

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THE existence of an intimate association between function and health is commonly accepted. Our profession frequently has under observation the results of excess in activity, whether physical, mental or digestive; suitable and often prolonged rest is nearly always embodied in the advice given. Less frequently lack of activity is the cause of trouble, as when sedentary workers, or the lethargic and obese, suffer for want of physical activity; here muscular exercise is prescribed.

But taken as a whole the profession is and has been so occupied with the arrest and cure of definite disease, with efforts at patching up the maimed, and, if possible, at restoring them to health, that study of the laws of activity upon which the maintenance of health is based and, incidentally, the prevention of most diseases, has received but slight attention.

Even that branch of the profession concerned with the public health has been so busied with eliminating the causes of such diseases as typhoid, typhus, plague, malaria, hookworm, bilharzia, tuberculosis, and infantile diarrhea, that the existence of a physiology of health has only received general recognition in the provision of city parks and playing centres, and the call for open windows and sufficient sleep, without any attempt being made to define how and when fresh air should be indulged in and exercise taken.

Modern methods of industrial productivity have, however, brought into existence a need for close understanding of the laws of activity. Both wage earner and wage payer desire to know under what circumstances productivity is at its best. During

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the World War the need for knowledge on this matter became urgent, since a maximum output of munitions was required from a minimum staff of workers and a limited amount of machinery, in order to supply the armies in the field. Long hours and continuous employment with no week-end rests, were at first resorted to with disastrous results. Investigations were then started, particularly by the Health of Munition Workers' Committee in Great Britain, purely for the purpose of increasing output; the health of the workers was entirely subsidiary, when the very existence of the nation hung in the balance. But the farther the inquiries were carried and the closer went the research, the more clear did it become that maximum production was a function of maximum health. So much was this so that the work then initiated was maintained when the war was over and placed on a sound basis by bringing into existence the Industrial Fatigue Research Board.

The name of this board is perhaps rather unfortunate, being reminiscent of its origin at a time when pathological fatigue was a feature of industrial work. The board exists today to study and lay bare the laws of human activity. Fatigue only comes into the picture as a diminution of productive capacity, such as is recovered from before work is resumed, such as is indeed required for healthy rest to be earned, and for the never resting pendulum of metabolism to swing from katabolic effort to anabolic well-being.

Some advantage may accrue from considering what light has been thrown upon the laws of health from various angles by these researches into industrial activity. In industry, particularly where repetition work is in progress, records of output are often available which present the investigator with invaluable material for making deductions as to the effect of various conditions upon productive capacity. Similarly records of labour turnover indicate the effect upon psychology or contentment; and of lost time, whether for sickness or accidents, upon health.

In practice output records are most often used. They are for the most part accurately kept in every factory, because wages are based upon them; moreover, whenever opportunity has arisen of

comparing optimum conditions for output with those for labour turnover and lost-time, invariably they have been found to coincide. Records of labour turnover and lost-time are not so usually kept, or if kept, are not so accurate. An important discovery, as yet far too little appreciated, is laid bare in the statement that conditions which are best for output, are best for labour turnover and lost-time; it is that economic production is a function of health in the producers. The objectives of altruistic philanthropy are found to coincide with the egoistical desires of industrial entrepreneurs. And some explanation is found of the rule that well paid labour is always cheap labour. When once industry is organized in reference to the physiological needs of the human machine, unrest and discontent, bred of ill-being, must recede into the background, giving place to the progressive activity of exuberant health. While at the same time, healthy energy will be given with economic efficiency in return for wages paid.

The lines of research followed may be reviewed by glancing at some of the results found to follow upon alterations in hours of work and use of rest-pauses, upon variations in atmospheric conditions of temperature and humidity, and in lighting, and upon modifications in methods of work.

HOURS OF LABOUR

Pronounced results followed reduction of over long hours during the war period; a few instances may be quoted.

a. Women turning fuse bodies when working 74.5 hours per week, gave a relative output of 108; when working 63.5 hours, the output gradually rose, during about three months, to 131; and when working 58.5 hours, it again gradually rose, during four months, to 168. In other words at this process, a heavy one for women, a reduction in hours of 16 per week, gave a 60 per cent increase in output.

b. Men sizing fuse bodies—a heavy and strenuous task—when working 67 hours per week, gave a relative output of 100. When working 60 hours, output steadily rose, during four months, to 120; when the hours were further reduced to 56, it rose, during the

next five months, to 139. Here 11 hours less work per week resulted in 39 per cent greater output.

c. Boys, on the other hand, employed at the light repetition work of boring top caps, gave a relative output of 100, when working 72.5 hours per week, which fell to 97 (even though the rate of hourly output rose to 129) when the working week was reduced to 54.5 hours. But even here the reduction in hours, 18 per week, or 25 per cent, was out of proportion to the 3 per cent loss of output experienced.

d. Another instance, chosen from pre-war experience, of heavy work, refers to men tending blast furnaces, first on twelve-hour shifts, and later on eight-hour shifts. Here an increase of some 18 per cent in output was finally obtained; but this level of output was not attained until some thirteen months after the eight-hour shifts were introduced in June, 1912.

Certain deductions emerge from such researches of which the instances just quoted are only samples.

I. The more the worker sets the pace rather than a machine, and the greater is the physical effort involved, the greater is the benefit to output of reducing excessively long hours. Not only does the rate of hourly output increase, but the total output for the fewer hours worked, is greater than that for the longer spell.

II. Workers tend unconsciously to expend the same amount of energy daily at their work; they may concentrate it in a short period, or spread it over a long period. Energy is normally expended in two ways; (*a*) some is expended every hour by merely attending the factory, and sitting or standing about without doing any work; such energy may be termed *static* energy; and (*b*) energy is expended when work is done; such energy may be termed *kinetic* energy. Two hours of static energy are saved daily when hours are reduced from ten hours to eight, and this amount of energy is then at the disposal of the worker to expend during eight hours as kinetic energy. It is this saved static energy which enables the worker actually to produce more articles in eight hours than in ten, without expending any more than his normal amount of daily energy.

III. When hours of work are reduced, the increased production

which may finally be expected, does not accrue immediately. Time apparently is required before the worker can adjust the rate of his energy-expenditure so as to consume what he has available. The lighter and less complicated the process, the shorter is the time required; thus the women turning fuse bodies only took from three to four months in attaining a new level of output when hours were reduced, while the blast furnacemen did not attain the full output on the eight-hour shift until thirteen months had elapsed.

In contrast, if hours are lengthened, say from eight to ten, the available energy has immediately to be spread over the added period, or over-fatigue must result. This means that the ten-hour pace is immediately adopted. As a matter of practical procedure the way in which to employ overtime in any period of rush is here indicated. Given that, where the eight-hour day is being worked, four hours of overtime are to be added during the week, a better result may be expected, from increasing hours on four days by one hour, from eight to nine, than by increasing hours on two days by two hours from eight to ten. In the latter case the ten-hour pace will promptly be adopted and tend to be maintained on the other four days also; and it will take some time for the pace to quicken again when the overtime hours are discarded. In the former case the nine-hour pace will be adopted and worked all the week, and a shorter time will be required for getting back to the eight-hour pace when overtime is discarded, than for getting back from the ten-hour pace.

Rest-pauses. In order that activity may be maintained from day to day and from week to week, rest must adequately balance exertion. This statement applies also in modified form to activity during spells of work. Workers may be observed interpolating short pauses from time to time. And in such heavy labour as road-making, dock work and agriculture, workers were noted to rest during about eleven minutes every hour and, if the type of work was uniform and not irregular, each rest averaged slightly over one minute and recurred after about six-minute intervals. For more arduous work, longer rests were found to be taken, even though the men were on piece-rates; 22 to 26 minutes per hour at pitch loading; 14 to 28 minutes at rolling red hot tinplates; and 7

to 22 minutes at coal getting, varying with the atmospheric conditions underground.

In some cases rest-pauses have been organized instead of being left to each worker's own feelings. Thus a five-minute rest per hour was given to girls assembling bicycle chains; their output thereafter steadily rose until at the end of about six months it was about 15 per cent higher than before the change.

Generally the custom is spreading in industry of introducing a rest-pause of about ten minutes in the middle of the morning and the afternoon when the spell of work is four hours or more. During this pause, light refreshment is also served.

The correct moment at which rest-pauses should be interpolated calls for scientific consideration. The output needs to be watched from hour to hour; when all is well, output should climb slightly as the day proceeds and the muscles settle to their task; there should be no falling away. The same should hold good for output from day to day throughout the week. If, however, the rate of output begins to decline before the end of the spell (and, if it does, the daily output will be found to begin to decline before the end of the week), all is not well; and the output may be improved by interposing a rest-pause at the moment when the rate of output begins to fall off.

An interesting observation has also been made that the value of a rest-pause may be increased by alterations in posture rather than by remaining inert during the pause. Such alterations as walking quietly instead of remaining seated, appear to act beneficially by modifying temporarily the course of the circulation; a similar result may ensue from taking light refreshment, apart from the energy value obtained from the food consumed.

ATMOSPHERIC CONDITIONS

Modern research into the aim and object of ventilation has been largely based upon investigations made under industrial conditions; it has entirely altered our outlook upon the matter. Today such alterations in the chemical composition of the air as occur in dwelling rooms owing to respiration, are recognized to be negligible. On the other hand, the influence upon the heat regulation of the

body exerted by atmospheric conditions, has been shown to be of fundamental import. The whole metabolic activity of the body is regulated by its capacity for surrendering heat to its surroundings; and this capacity depends upon the cooling power of the air. Cool air in contact with the body will abstract heat; if this cool air be moving, it will exert an effect greater in proportion to the rapidity of its movement. When the skin, as is usual, is sweating, dry air evaporates the sweat rapidly and so cools the body rapidly; if the air is already charged with moisture, it cannot evaporate sweat and so cannot cool the body in this way. For the skin to be able to excrete sweat, it must have a free supply of blood; when sweating is copious and free, a strain is thrown on the heart to maintain in the skin a sufficient supply of blood. In industry this strain occurs just when the muscles are calling for a full supply of blood to enable them to perform work. Visible sweating represents heart strain which should, whenever possible, be obviated by improving the cooling power of the air. Ventilation is thus found to be bound up with the physical conditions of the air,—its warmth, humidity and rate of movement.

Observations have shown how in heavy and hot work, output falls in warm weather and rises in cool weather; thus, in charging blast furnaces, it varied from 88.5 in summer to 105.0 in winter; in rolling mills it varied from 96.5 to 105.2; and in tinplate mills from 96.9 to 103.3. Such output with exposure to radiant heat can be, and has been, directly improved in glass bottle-making and in tinplate-rolling during hot weather by submitting the workers to cold air douches.

Output at less strenuous and hot work is found to vary with temperature, depending somewhat upon the amount of energy required. The greater is the physical activity, the lower should be the temperature in order to command the best results. For work of medium activity, the optimum temperature is somewhere about 65°F.

This optimum is found to coincide with the optimum temperature at which fewest accidents occur; while, as the temperature rises above or falls below this optimum, the accident frequency

rises, rapidly as the temperature rises, more slowly as it falls; thus, if 100 represent the accident frequency at the optimum temperature, it was found to rise to 130 at 10° higher and at 15° lower.

The tendency to experience lost-time for sickness also varies with temperature, and appears to have the same optimum as that determined for accidents and production.

It can hardly be a coincidence that "below 65°F., moist air feels cooler than dry air because its conductivity is raised, and up to this temperature evaporation is not an important factor in cooling."

The effect of warmth and humidity has been particularly studied in the textile industries, where threads work best and break less, when kept in a warm, moist atmosphere, in an atmosphere, indeed, which places the workers at a physiological disadvantage. Here best output is attained as the result of a compromise,—at about 73°F.,—a temperature which is lower than the optimum for the threads, but higher than the optimum for the workers. Above this temperature the output is found to fall away in the afternoon as compared with that of the morning, and to give for the day a total lower output. Advantage, however, has been gained by placing the air round the workers in movement, by using a simple paddle fan. This scheme cools the worker without disagreeing with the threads, and is advantageous to output.

Lighting. Throughout these studies the intimate way in which the human body is found to react to its environment cannot but challenge attention. In no case is the reaction closer than with regard to illumination. The way has been observed in which output in the textile industries increases week by week as the hours of daylight lengthen in the spring, from a figure of 43 per week for mid-winter to one of over 50 for twelve weeks later, and for the same hour of the day from figures of 80.3 and 83.9 for the first and last (i.e., the darkest) hours respectively of the working day in mid-winter to figures of 86.7 and 87.0 for these hours of the day twelve weeks later.

The observation is a familiar one that in temperate climes sick-

ness and mortality are high in winter and low in summer, a fact, which, although influenced by other climatic effects, such as temperature, is to no small extent due to light.

A more intensive study, made in the printing industry, has brought out the effect of illumination upon the quantity and quality of the work of compositors, which was found not to attain the standard under daylight conditions until an artificial illumination was obtained giving over 20 foot-candles of light on the work.

Different processes call for different standards of light; but when illumination falls below a certain minimum standard, the capacity of the eye for adaptation fails. Work in coal mines affords the most striking instance in point; here the light from safety lamps falling on the black light-absorbing surface at the coal face, only averages 0.018 of a foot-candle. Prolonged exposure to such conditions induces miners' nystagmus, that curious and distressing occupational neurosis associated with uncontrollable oscillation of the eyeballs. This disease is hardly ever encountered in coal mines illuminated by naked candle-lights, even though such illumination only amounts to 0.09 of a foot-candle.

METHODS OF WORK

References have so far been confined to a few examples of impersonal factors which influence health, activity and industrial efficiency. Of equal value are other factors, which may be grouped together under the heading "methods of work." They include: (*a*) fitting workers, physically and mentally, to the work for which they may be suited. Personalities vary. Probably everyone is particularly suited for something; thus a man with an artificial hand will be exposed to less risk than a man with two hands if employed at a hand-fed power-press; deafness may be an asset to a calculating clerk in a noisy office. Only by allowing for the characteristics of each person, can every wage earner be given opportunity to develop his capacity. Scientific methods of selection, which are still in course of elucidation, must replace trial-and-error methods of haphazard engagement of labour; only so can the tendency be minimized for labour to drift from factory to factory in search of more congenial environment, the tendency

which expresses itself as labour turnover, one of the greatest economic burdens which modern industry has to bear. By such means labour turnover, which today generally averages about 100 per cent per annum and may even reach over 400 per cent, can be, and has been, reduced to below 10 per cent. When mated to his occupation, the worker not only works better, but he works more happily and contentedly, and experiences fewer accidents and less sickness. (*b*) Devising machinery so that it is well suited to the workers. The provision of platforms for short workers to stand on, and of seats, adjustable in height, comes under this heading; as does also the maintenance of the supplies of material conveniently arranged for manipulation and for subsequent removal after manipulation. The American designed frame for bobbin-winding in cotton mills, as compared with the older English frame, is a good example of design modified to lessen the reach and stoop of the workers. The mere provision of suitable seats so that workers could sit or stand as they liked while buffing forks and spoons, led to increases in output which amounted in two instances to 23 and 32 per cent. (*c*) Teaching workers how to use their limbs and apply force economically. The need of instruction on such lines is only slowly coming to be appreciated in industry. The example set in sports, where we submit to instruction, as to how to play golf or to row, how to box or fence, has not yet been followed in our factories and mines. Yet efforts in this direction give satisfactory results; thus only one week of instruction to a dozen workers employed upon roughing metal spoons, was followed by 29 per cent increase in output; the result was obtained by simplifying motion and eliminating unnecessary effort. Coal miners, when persuaded to adopt in the use of their picks, the rhythm of stroke kept by their more skilled companions, found that their output increased by some 15 per cent. A study of how to carry burdens,—a process as old as the history of *homo sapiens*,—has been made, in which the physiological cost, as measured by respiratory exchange, of different methods of carrying was determined. The results indicated that the cost of weight-carrying depends on the degree of displacement of the body necessary to bring a centre of gravity over the feet. The

less the displacement, the cheaper the cost. Thus, carrying by a yoke on the shoulders or by bundles held in the hands, which call for no displacement, are the cheapest methods.

Only some of the factors which influence daily activity can be studied by means of industrial records. Other factors connected with life as lived outside working hours, must exert their effect. In other words, industrial investigations can only be expected to point the way to laws of activity. They cannot reveal them perfectly.

Even presuming that knowledge regarding the laws of activity become more clearly and plainly understood, objection may be raised that, by subjecting the human frame to organized and even stereotyped conditions of activity, it must be converted into an unintelligent automaton. But such an objection is invalid. What is attempted is, by removing obstacles from the natural rhythm of activity, to permit fuller play to healthy life and enjoyment. Joy results from pleasurable activity and endeavour; only irritation and discontent result when the rhythm of activity is being continually broken and hampered.

When we understand better, from continuation of these studies into industrial activity, the optima for hours of work, conditions of labour, and methods, we shall understand better how, by maintaining health, to avoid disease, which only too often is the result of long continued disorder in function.

ORGANIC CHEMISTRY. ITS RELATION TO MEDICINE[1]

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THE ART OF HEALING, AND SCIENCE

WILLIAM HARVEY, whose memory your society honors, emphasized in his works the importance of systematic observation for medical science. The achievements of this genuine and great scientist depended upon experimental method. His contemporary, Francis Bacon, whose personality and significance, unlike Harvey's, is widely contested, had views on science which according to Harvey more befitted a Lord Chancellor than a philosopher. Yet many perceptions of Bacon, Lord Verulam, deserve consideration even today. It is he who declared: "Let no one hope to guide or manipulate Nature who does not thoroughly understand her."

The general philosophical foundations of medicine were thus already laid several centuries ago. It was furthermore the same philosopher Bacon who defined in a way still applicable today the various tasks of medicine: the protection of health, the healing of sickness, the lengthening of life, the alleviation of pain.

But the development of chemistry and physics and other branches of science had until the last century been so slow that a hundred years ago medicine as a science was also in but a slightly developed, unsatisfactory state. As late as 1836 the famous Hufeland declared opium to be the only remedy for malaria. A turning-point at this time was the publication of Johannes Müller's "Lehrbuch der Physiologie." The matronly countenance of Lady Medicine, as Helmholtz once remarked, was made fresh with life at Science's Fountain of Youth. Great progress resulted from Laënnec's introduction of stethoscopy for the diagnosis of

[1] Lecture delivered April 9, 1927.

internal diseases, and from the foundation of pathological anatomy as an independent medical discipline by Laënnec in Paris, Rokitansky in Vienna, and Virchow in Berlin. The investigation of the putrefactive and fermentative processes, the development of bacteriology, of vaccination, immunology, serum-therapy and chemotherapy in the life-works of Pasteur, Koch and Ehrlich, brought about the great advance in the medical sciences.

To raise the question, whether science, whether more particularly organic chemistry, has had, and has, an essential influence on medicine would at first blush seem to be breaking open an unlocked door. Especially in a country where medical education has reached such great heights as is here the case. Indeed we ask the question whether medicine is a science; and the question, what the proper relation between science and medicine is; and the question, how great a proportion of scientific method and scientific instruction the medical educational course should contain. In Germany these questions are not only raised at present; they are also heatedly debated. A noteworthy lecture on "Heilkunst und Naturwissenschaft" by my friend Sauerbruch, the Munich surgeon, at the last meeting of the German Association of Scientists and Physicians, treated the present antitheses of our respective views. Sauerbruch is strongly in favor of burdening the physicians less with scientific method, in order thus the more to bring them back to the simple bedside observation of patients. In doing this one must, of course, not forget that very great domains of medicine, such as the treatment of epidemics and tropical diseases, the prevention of infectious diseases, antitoxin treatment, and so forth, are created by exact scientific research and consist of strictly scientific method. These are domains of medical science. But there is another kind of medicine, namely the activity of the general practitioner at the bedside. This domain is not chiefly science; it is composed more of art and experience. The individual general practitioner neither is, nor should be, a scholar or a technician working with scientific methods. Not merely by means of exact method, but much rather with his whole soul, his entire character, should the physician carry out his difficult profession. Scientific method should not be permitted to

render mechanical and schematic the physician's activity, nor should it be allowed to displace the direct and penetrating observation with which he espies and explains the disease. Scientific methods cannot replace the intuition of the physician; they can merely complete it. Sauerbruch extols this amazing faculty, and he regards intuition as a form of instinct, improved by human evolution. Intuition, however, is perhaps a still greater complex of qualities and faculties. In the intuition of the physician, I see an inborn gift for rapid and accurate perception, combined with acquired, live medical experience. Intuition is, thus, a result of observational talent and medical memory. It is, to be sure, not the sole function of our medical schools to educate the born supreme and leading physician, nor can our medical students be expected to bring any capital of experience into the schools with them. In order to educate the thousands of practicing physicians which the people need, the elements of scientific training and scientific method are indispensable. But they should not displace the observation of sickness. Rather they should deepen this observation. The state of medicine will always be dependent upon the state of physiological knowledge; the state of therapeutics depends upon the methods discovered for healing and the available medicinal agents.

Hence chemistry is indispensable in medical instruction, organic chemistry particularly, not merely because it is the gateway to physiology, but also for the foundation of modern therapeutic methods. But only to a limited extent, we must admit, can chemistry and other scientific disciplines serve the purposes of the practicing physician, whether by giving correct conceptions of the vital processes or by the development of precise methods of chemical diagnosis. In fulfilling his function of bringing alleviation and healing, the practicing physician can use but a small measure of chemical knowledge and training. But more important and far-reaching indeed is it to create new methods of healing—at one stroke to bring protection and healing to thousands and millions of human beings. Scientific medicine achieves this by coöperation with science, particularly with organic and physiological chemistry. The individual physician's powers at any

given time are limited only by the contemporaneous status of scientific medicine. And it is by virtue of its connection with general scientific research that scientific medicine has raised itself to its present standard of achievement and will continue further to perfect itself. Organic chemistry, above all, is intimately bound up with scientific medicine, for it is organic chemistry which acquaints us with the whole range of materials of which the human body is built, from its main constituents to those present only in traces, yet indispensable. Organic chemistry seeks to make clear the individual processes in the living body, such as the metabolism of food stuffs, the course of the oxidation processes, the processes in the contracting muscle, the dependence of the various separate functions on the internal secretions, the enzymatic reactions. Indeed the life of every single cell is a complex of carefully regulated and inter-related enzymatic reactions.

DEVELOPMENT OF ORGANIC CHEMISTRY

Organic chemistry had its origin at the beginning of the last century in the science of living or organized bodies. Somewhat earlier still, in the works of T. Bergman, we are told: "The organic bodies fall into two well-defined classes—the plants and the animals." Berzelius, in his "Tierchemie" of 1806, was the first to denote by the term organic chemistry that part of physiology which deals with the composition of the living body together with the chemical processes occurring within it. In his famous "Lehrbuch der Chemie" (1812) he distinguished between mineral and organic chemistry, which latter describes the intimate structure of plants and animals along with the chemical processes of which their life consists. Even the first analytical researches of Berzelius made it clear that inorganic and organic bodies have the same constituents and obey the same chemical relationships—laws of affinity—and thus are continually changing into one another. These experimental investigations, which were further continued mainly by means of the analyses of Liebig and the organizing theories of Gerhardt, clearly showed that the element common to all organic substances is carbon. Consequently dur-

ing the first half of the last century the concept organic chemistry developed in Gmelins's Handbook simply into the chemistry of the carbon compounds, and this limiting definition was accepted and promulgated by Kolbe, Kekulé, and Butlerow. The earlier organic chemistry then became physiological chemistry.

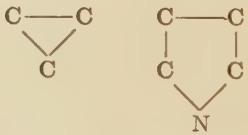
While this demarcation of inorganic and organic chemistry became valid, there continued between the two great domains a wall of theory. This was erected by a belief in a peculiar vital force which had first been assumed in Paris by Bordet, a physician. According to Berzelius, the building up of organic substances was supposed to be a prerogative of organized nature. The peculiarity of living bodies and of organic compounds was supposed to have its origin not in their respective elementary constituents, but rather in the absolute necessity of a special life-force. Berzelius laid down the following principle: Human art is incapable of uniting the elements of inorganic nature after the manner of living Nature. But this point of view, which was shared by all save a few contemporaries such as Berthollet and Oersted, is based on a faulty separation of the respective ideas of living body and organic compound. For such things as blood corpuscles, nerves, and blood vessels were in the beginning included in organic chemistry.

In order to banish the vital force from the explanation of the organic compounds, and to penetrate more deeply into organic chemistry, a theoretical and an experimental step forward was necessary. The organic substances, the carbon compounds, had first to be sharply distinguished, as a concept, from the organized structures of the plant and animal kingdoms, and then it was necessary to open the way to the synthesis of the substances produced by the living organism. This was first achieved in the urea synthesis of Wöhler (1828) and then in Kolbe's acetic acid synthesis, and in the systematic syntheses of Berthelot.

The distinction between organic and inorganic chemistry has been retained chiefly because the carbon compounds are so extremely numerous, a fact which has a special cause and gives rise to special problems. In 1883 there were as many as 20,000 compounds known; by 1906, 180,000 substances, mostly synthetic, had

been analyzed and investigated. Today the number far exceeds 200,000.

The cause for the fact that among the known 90 elements the single element carbon forms more compounds than all the others together, rests upon a particular property of carbon, namely the great ability of its atoms to unite each other. Thus chains of carbon atoms arise; straight ones, C−C−C−C, and branched ones, , combined with hydrogen, oxygen, nitrogen, and other elements; and furthermore there arise rings of carbon, some composed only of the latter, others having as members, besides carbon atoms, also atoms of other elements such as nitrogen and oxygen:



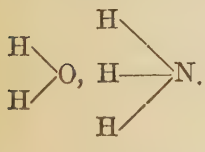
These chains and rings are remarkable for their extraordinary stability, which is not to be reproduced by structures of any other elements. The chains can contain atoms to the number of 10, 20, and many more. As for the rings, it was generally supposed until last year that their limits are reached sooner, with 8 or 9 links, carbon atoms. But recently an investigation by Ruzicka on musk and similar animal scents has shown that rings with for instance 17 carbon atoms occur in Nature and can be synthesized.

A consequence of this combining ability is the occurrence of different compounds of the same molecular formula. Following Berzelius we call this phenomenon isomerism. It is very rare among inorganic substances, prodigiously frequent among organic ones. If we look up in our indices (of the year 1910) a fairly simple carbon compound, $C_6H_{10}O_5$, we find 113 substances with this formula. They are all different one from another. Should we select a more complex formula, $C_{10}H_{13}O_2N$, we should find not fewer, but still more isomers—175. In his Harvey Lecture of the year 1912 Th. W. Richards explained this phenomenon by

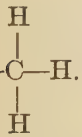
reminding us how different words may be formed out of the same letters by different arrangements, for instance, "art" and "rat." Now, here it is but three unit particles, letters of the alphabet, which are being compared to the unit particles of the elements, their atoms. But the unit particle of cane sugar, its molecule, is built of 45 atoms of three elements. And this is still a fairly simple compound.

Therefore, in order to explain the nature of a substance, it is necessary to determine the mode of connection of its elementary particles, its constituent atoms. This is the structural chemistry of Kekulé. This structural determination alone, however, is not sufficient. For the representation of structures on paper is not unambiguous; it must still be supplemented by more exact spatial conceptions. This was achieved by the stereochemistry of van't Hoff and Le Bel.

According to the views of Gerhardt, organic substances were compared with some simple inorganic types, for instance H—Cl,



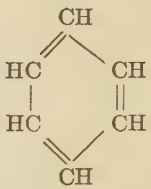
In 1857, Kekulé added to these types a further



one H—C—H. Yet more important was it that he explained

the actual meaning of these types by a more fundamental assumption: there are certain valence numbers, units of chemical force, proper to the elements; these are thus monovalent, di-, tri-, tetravalent. The force operating in compounds thus consists of a certain number of partial or unit forces, namely the single valences of an elementary atom. On the basis of this theory Kekulé declared it to be an objective of organic chemistry to determine the structure of the molecules. Thereafter and until today this has been a principal task of organic chemistry. Knowledge of structure is the condition for synthesis.

One of the most important single cases of structural chemistry was benzene, a hydrocarbon of the formula C_6H_6. After Faraday's discovery forty years elapsed before the nature of benzene was explained by Kekulé by means of the famous formula

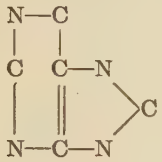


This theory inspired the evolution of a great class of derivatives. Among these were numerous medicaments, dyes, explosives, perfumes. During a certain period the principal material source for the development of this so-called aromatic chemistry was coal tar. Plant and animal products diminished somewhat in importance, until near the end of the last century when research under the leadership of E. Fischer again directed its attention more towards the great problems which Nature sets, the solution of which is important for physiology and medicine.

Chemistry of the natural products

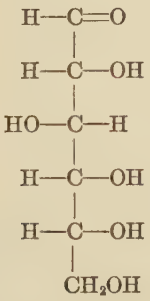
Of the classes of compounds physiologically important, the fats alone were cleared up in the youthful days of organic chemistry by Chevreul, at least as regards their salient features. But in every problem of chemistry the solution has been arrived at in many stages only through the labor of several generations. The purine group, which includes physiologically important substances such as uric acid, xanthine, caffeine, presented a structural problem which, compared with the tasks of today such as thyroxin, insulin, and the vitamines, is remarkably simple. The first masterly investigation by Wöhler and Liebig in the years 1837 and 1838, and a second one by v. Baeyer in 1863 and 1864 were only preparatory to the actual determination of the constitution. This goal and the synthesis were achieved for the first time by Fischer in two great researches, 1881–84 and 1894–1900. And

what was the prize which had been striven for so long? Merely a chain of 3 atoms of carbon: C — C — C, coupled, to be sure, with 2 molecules of urea into a skeleton of the formula

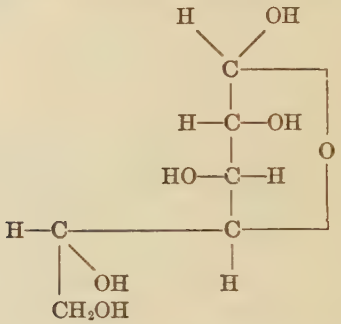


The era of Emil Fischer also brought about our enlightenment on the subject of the carbohydrates, as regards the main features at least of the simplest sugars. The so-called high-molecular ones, such as starch and cellulose, still present great problems in our own time. But also in connection with the simple sugars, the monoses and bioses, an abundance of new significant problems has been disclosed by the researches of the school of St. Andrews, and in this country by those of Levene and of Hudson. Even the generally known results of Fischer on the nature of glucose, which were seemingly final, are continually demanding further improvement and refinement to which medicine, since the discovery of insulin, has been anxiously looking forward.

Fischer's formula for grape-sugar was originally that of an aldehyde:



But the properties of glucose and its relations with its naturally occurring derivatives the glucosides subsequently necessitated a modification of the formula suggested by Tollens, according to which the terminal carbonyl group C = O enters into combination with one of the alcoholic groups of the same molecule to form an acetal-like ring structure, a lactal structure:



Finally, according to knowledge acquired in recent years, glucose seems to occur in the blood in a form essentially different from its usual variety due to the action of the insulin in the blood. Banting and Best and their co-workers have done chemistry and medicine an unforgettable service by their discovery of the hormone of the pancreas. The action of insulin is regarded as probably due to the transformation of the ordinary stable form of glucose (1,4-glucose) into a different variety, γ-glucose, characterized by a different anhydride ring. This is a more unstable, more reactive form, and the only one which suffers oxidation in the living organism. Therefore a dearth of insulin must result in glucosuria: the ordinary form of glucose accumulates in the blood, and is consequently eliminated in the urine. When insulin is then artificially supplied, the blood-sugar even of diabetics goes over into the form necessary for the body, the γ-form.

The great series of investigations on the proteins and their constituent units, which E. Fischer carried out during the last decades of his life, together with the researches of Kossel, Hopkins, Dakin, Levene, and other scientists, have brought to light the fundamental features of the constitution of this group too.

Numerous individual polypeptides and amino-acids were proved by analytical methods to be constituents of the protein molecules and were brought within the reach of synthesis. We now know the fundamental linkages between the constituents of the protein molecules to be of an amide nature $-\overset{\overset{\|}{O}}{C}-NH-CH_2-$.

But as regards finer details, the chemistry of the proteins has remained incomplete.

The poisonous and curative substances isolated about a century ago from plants have been a great source of inspiration to chemistry and pharmacology. At the same time as Davy was isolating the elements potassium and sodium from the inorganic alkalies known of old, other workers were beginning to extract from plants alkali-like organic substances, strange nitrogen compounds of complex constitution, such as morphine, quinine, atropine, cocaine, nicotine, and others. The imagination of savants could not have conceived combinations of atoms as strange as those which analysis reveals in these alkaloids. To be sure, even after more than a hundred years, after the labor of generations, the constitution of morphine and its derivatives, for instance, is not yet determined in all particulars. But it is to the efforts of the chemists to synthesize these plant bases or to find substitutes for them that we owe a great enrichment of our store of drugs. A few examples of such syntheses of medicinal agents will best indicate the progress in which the coöperation during several decades of organic chemistry and medicine has resulted. This is the road which gradually leads from the method of pure speculation in the preparation of medicine to the new science of chemotherapy.

Synthesis of medicinal agents

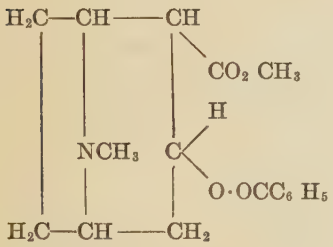
In the early days of the synthetic production of medicines, systematic work planned on a generous scale was lacking. The first artificial antipyretic, antifebrin, was quite a simple derivative of anilin, acetanilide. It was discovered through its being accidentally confused with naphthalene. A greatly improved preparation, phenacetin, appeared shortly afterwards, as a result

of the efforts of industry to utilize a hitherto worthless by-product, p-nitrophenol. At that time, in the eighties of the last century, people were looking for substitutes for quinine which would be still more effective. The structure of the quinine molecule is so complex that no results of practical value were expected of a possible synthesis. Therefore chemists tried to "imitate" it (that is, to produce a substance of similar therapeutic properties) by means of simpler atomic groupings. In those days quinine was supposed to be what in reality it is not, namely a hydrogenated derivative of quinoline

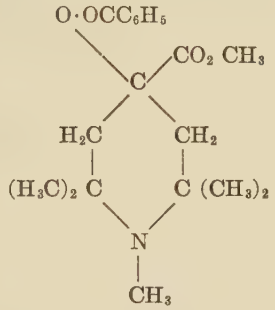
For this reason derivatives of hydrogenated quinoline and of quinoline proper, such as kairin, kairolin, thallin, analgen, were sought out: these actually did have antipyretic action which was, however, counteracted by various detrimental qualities. Phenyl-dimethyl-pyrazolone, discovered through synthesis by Knorr, and first applied to medicine by Filehne, was also at first supposed to be a quinoline derivative; this likewise is in reality not the case. Nevertheless, under the name of antipyrin, it proved to be an excellent remedy against fever. And it furthermore gave rise to the discovery of still better quinine substitutes such as pyramidon.

The case of anesthetics furnishes another clear proof of the success and usefulness of the chemist's efforts. Here it has been one of his objectives to make simplified "imitations" of cocaine, likewise a model set by Nature. Cocaine, an amino-acid ester was supposed—incorrectly—to be a hydrogenated benzene or pyridine. A synthesis by Merling resulted in the hydrogenated pyridine derivative, eucaine; another product first synthetized by Einhorn is novocain, an ester of amino-benzoic acid, important as an anesthetic, and at present competing with cocaine in many applications. Fourneau finally has still further simplified the

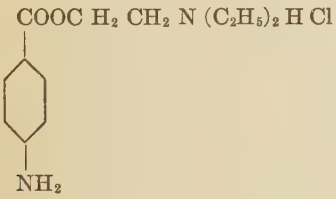
molecule by means of an aliphatic combination of groups, stovaine, containing the essential constituents of the more complex cocaine.



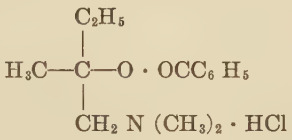
cocaine



α-eucaine



novocaine



stovaine

The real, complete synthesis of naturally occurring l-cocaine itself was for the first time successfully achieved but a few years ago, and what is more, in a way, as it seems, applicable on a large scale. This synthesis, furthermore, resulted in the artificial building up not only of native cocaine, but of a series of very closely related alkaloids as well. These have the same structure as cocaine; they are stereoisomeric with it, an interesting subject for pharmacological comparison. The pharmacological value of these stereoisomers is, according to the researches of Gottlieb, quite unequal. The best of them, psicaine, is in Gottlieb's judgment more intensely active than cocaine and at the same time much less poisonous. It has not the dangerous euphoric and intoxicating action of cocaine. The fact that psicaine can be produced synthetically in any amount puts into the hands of the governments of the various nations the opportunity, without loss

to medicine, at one blow to wipe out cocaine and the cocaine abuse the world over—if the governments wish it.

In the last decades synthesis has furnished such a wealth of medicines that physicians are overwhelmed with a mass of preparations and advertisements. The selection of the good preparations from among the many offered is growing increasingly difficult. The commercial exploitation of new medicaments is certainly detrimental. Nevertheless mankind will receive lasting benefit therefrom. For of the numerous competing remedies, only the most serviceable will in time remain, whereas the rest will be gradually crowded out to disappear again. The exertions of synthetic chemistry will eventually furnish the physician with a complete gamut of good anesthetics, antipyretics, analgesics, antiseptics, hypnotics, narcotics, etc.

Chemotherapy

Most medicaments seek to influence the symptoms of the various diseases, more exactly, they seek to influence the human organism. In contradistinction to this symptomatic medicine, there is causal medicine, which directs its attack against the causes of disease, its provokers. Part of this new movement is chemotherapy, founded by Paul Ehrlich, who was at once physician and chemist.

Ehrlich defined the task of chemotherapy as the systematic seeking out of remedies which act specifically against the parasitic microörganisms, the causes of disease, without injuring the human body, the body cells and fluids. The slogan of chemotherapy became: learn to aim chemically. The substances sought by synthetic methods were to be only parasitotropic, as little as possible organotropic. Their action in the organism must be directed selectively against the parasites. For protective substances which pass into the serum and are capable of successful transference to other individuals are not formed in the case of all infectious diseases. Such protective substances are generally absent, for instance, in most protozoan infections. The strict definition of chemotherapy seems to exclude the therapy of ma-

laria with quinine, the therapy of amebic dysentery with emetin. For here one is working with natural remedies, not with synthetic ones. But because of the nature of the therapeutic method there is really no difference between these cases and the combating of malaria with plasmochin and the treatment of syphilis with salvarsan. But in contradistinction to the small number of naturally occurring remedies of sufficiently great selective action, chemotherapy carries out experiments with great series of active substances. Those are then selected which show the most favorable ratio of parasitotropic to organotropic qualities, that is, the most favorable therapeutic index.

Strictly speaking, it is impossible rigorously to define the boundary between symptomatic and causal medicine. It was a great inspiration of Ehrlich's, by means of the internal disinfectant (*Therapia sterilisans magna*), completely to exterminate the parasites. This idea is of heuristic value even though it is only partially substantiated by the facts. The synthetic agent is supposed to become fixed by means of certain binding or adhesive groups, the haptophores, to adsorptive components of the parasites, the chemoceptors; then the toxophoric group of the substance is supposed to exert its destructive influence on the parasite. But chemotherapy discloses cases of yet another mode of operation, where the stimulative action on the organism proper is the essential phenomenon. In this case the specific action consists of a stimulation of antibody formation (*Ictus immunisatorius*). There are, furthermore, cases such as, for example, plasmochin, which depend not upon a destruction of the parasites but only upon the checking of their successful progress. Here the chemical remedy brings about only a molestation and obstruction of the parasites. This knowledge opens up great opportunities for the combination of therapies, such as salvarsan with bismuth, germanin with antimony compounds, plasmochin with quinine.

Indeed, I am unable to discern a clear difference between the synthesis of medicines in the year 1882 (kairin) and the chemotherapy of 1926 (plasmochin). For the new plasmochin also

belongs to the class of the simplified variants of quinine, whose formula can be derived from the principle:

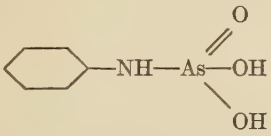
And the chemotherapeutic agents of Morgenroth such as optochin, eucupin, and vucine, are likewise formed by the remodelling of quinine. They are hydrogenated derivatives of its series. But how beautifully has the pharmacological method been perfected! For the discovery of plasmochin, reliable methods for quantitative measurement were contrived through the adaptation of avine malaria to the laboratory experiments. The cooperation between chemists and medical men has been perfected during the same period, although the more distant past does present us with a few examples of excellent coöperation, such as between Fischer and v. Mering, Knorr and Filehne. Nowadays, however, pharmacology no longer investigates single preparations, but rather systematically traces entire great series of variants. The goal is knowledge of the relations between chemical constitution and physiological action or chemotherapeutical value: to be sure in all fields we are as yet far removed from it.

It is the infectious diseases which set the great tasks for chemotherapy. The provokers are pathogenic bacteria, such as streptococci, staphylococci, pneumococci, tuberculosis bacilli. In other diseases they are spirochaetes, as in the case of syphilis and relapsing fever; in yet others they are protozoa, as in malaria and the trypanosome infections.

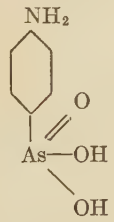
The successes of modern chemotherapy are conditioned by the union of medicine and organic chemistry. This cannot be shown better than by the history of salvarsan, which I should like to recall in a few words. But it is neither task nor intention of mine to speak to you about new medicaments and the results of their trial. In these things you are no doubt better informed than I can be.

Ehrlich's starting-point was atoxyl, which according to Uhlen-

hut and others showed remarkable activity against spirochaete and trypanosome infections. The atoxyl of Béchamp had been known since the year 1863. Had the formula

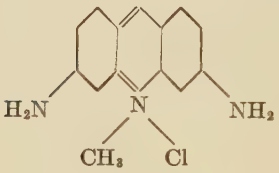


originally assigned to atoxyl been correct, then atoxyl could never have acquired importance as the foundation for chemical variations and syntheses. Ehrlich, the chemist, recognized that its structure had been falsely interpreted. He found in 1906 that the arsenic is in combination with the benzene nucleus:

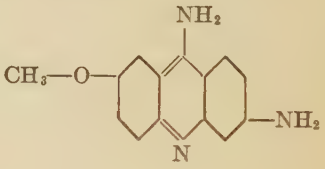


From this it followed that the arsonic acid residue might be transformed in the same manner as a nitro group. The reduction-products, and the analogues of the azo dyestuffs obtained from them, showed extraordinary activity. Of decisive importance for this increased activity was the transformation into compounds containing the more reactive trivalent arsenic. Ehrlich, the physician, studied the extermination of spirochaetes by great series of synthetic preparations, at first in test-tubes, and then in living animals. That is the procedure of chemotherapy. The result was "Ehrlich and Hata 606," salvarsan. On this foundation there has in the meantime arisen a great series of important aromatic arsenicals, such as neosalvarsan, silversalvarsan, spirocid or stovarsol, Albert 102. That the powerful stimulus having its roots in Ehrlich is bearing fruit in this country we see in the tryparsamide of Jacobs and Heidelberger of the Rockefeller Institute for Medical Research.

Dyestuffs excel particularly in the power of being fixed by the cells of parasites. Observations on the dyeing of living cells were Ehrlich's springboard. The introduction of methylene blue and gentian violet as well as the combination of dyes with metals as in mercurochrome have brought Young and Hill and other American chemotherapeutists great success. In Germany Benda and Morgenroth introduced at the same time, respectively, the dyestuffs trypaflavin or acriflavin, and rivanol



Trypaflavin



Rivanol

as efficacious means of internal disinfection.

But just as dyes are fixed, so can we also fix colorless substances, alkaloids, for instance, by adsorption upon animal fibers, wool and silk. Colorless analogues of the dyestuffs thus belong today among the most powerful weapons of chemotherapy. A complex aromatic urea derivative with sulfonic acid groups, consisting of many dyestuff-like coupled rings, is the remedy against sleeping sickness, Bayer 205 or germanin. It opens the way to an unsuspected new class of chemotherapeutic agents. The systematic discovery of this remedy shows the progress in method during the period from antipyrin to germanin.

Methods of enzyme research

Great as the successes of chemotherapy are, the achievements of the organic chemistry involved in it offer nothing new in principle. Fundamentally new chemical methods are on the other hand being developed among the groups of those substances indispensable for the functions of the organism.

The internal secretions, the hormones, appear in the greatest

dilutions, and are distinguished by so powerful an activity that only the best chemotherapeutic agents are to be compared with them. In this respect they are of a kind with the vitamins, which present similar problems. A peculiar feature of the hormones is that in the unrefined state in which we have them, in the form of the dried glands, they show a variable activity, due, it is often assumed, to the action of accompanying enzymes. More probably, however, their action is effaced by retarding substances. It is hence a great problem for chemical art, to increase the degree of purity of such physiologically active substances, and to obtain them in a homogeneous state. It is to be concluded from the experience gained formerly with adrenalin and recently with thyroxin in the investigations of Kendall, and later in those of Harrington and Barger and at the same time of Dakin in this country, that here we are dealing with stable and not over-complex compounds. In other cases, with which Professor Abel's Harvey Lecture dealt a few years ago, in the case of Banting's and Best's insulin, of the hormones of the pituitary gland, also in the case of a hormone of the female sexual organs, and in the recent case of an active principle in the liver against pernicious anemia, investigated by Minot and Cohn, even where there are as yet no pure substances on hand, the concentrations already obtained are very considerable. Here, also, one is dealing with substances of comparatively great stability. From the isolation of such hormones it is still a long way to the isolation of the active substances of the sera, the antitoxins, as well as the toxins. It may be that the investigations on ferments or enzymes during recent years will furnish a certain amount of stimulus and aid towards the solution of this problem. These researches concerned mainly such enzymes as the ones which split the proteins, the fats, and the carbohydrates in the living body, and those which carry the oxygen.

Until very recently, it was uncertain whether the agents causing the enzymatic reactions in the living organism are special organic compounds of high molecular weight or merely substances already known for a long time, present in particular colloidal states, in special conditions of dispersion. This question has

been decisively answered by the fact that in several cases investigated it has been possible to increase the enzymatic concentration, that is the degree of purity of the enzymes, to an extraordinary extent. In spite of the fact that the action of the enzymes is extremely dependent upon the accompanying substances and the experimental conditions, in some cases also upon their own state of division, methods could nevertheless be created quantitatively to follow the action of the enzymes, and hence their quantities and concentrations, throughout the whole range from the living cell to the purest preparations. In this way the enzymatic action could frequently be kept practically constant. But in doing this the enzymes were forced to undergo such variations in degree of dispersion that it is no longer possible to make the colloidal state responsible for their catalytic action.

The difficulty to be overcome in this field is that chemical means for the isolation of these strange bodies are out of the question. Chemical treatment leading to their separation is lacking. The method which proved capable of being worked up to considerable efficiency is that of adsorption by means of certain substances of powerful surface action, such as alumina and kaolin. The action of the adsorptive agent is here so highly specific and selective that it is possible to separate from each other the three chief enzymes of the pancreas gland: lipase, which splits fats; trypsin, which hydrolyzes proteins; and amylase, which breaks down starch. The selective action of certain adsorptive agents has been further developed to such an extent that we are able to separate enzymes from their activators (trypsin from enterokinase) and retarders and also quantitatively to part closely related enzymes, for instance the enzyme splitting saccharose and that splitting maltose (saccharase and maltase), the proteolytic enzymes trypsin and erepsin, and others. The possibility of preparing individually the enzymes occurring mixed in Nature and of applying them, for instance, to the hydrolysis of the proteins, promises to initiate a new development in protein chemistry. Henceforward we shall obtain well-defined, chemically homogeneous products as stages in the partial splitting-up of the proteins and thus be able to lay clear their individual complexes and atomic

groups for the analysis and isolation of which the older methods were inadequate. The application of the new method of selective adsorption promises results of practical value for medicine in the investigation of sera, in the isolation of the specific antitoxins. In this direction, Avery, Heidelberger and Goebel of the Rockefeller Institute have already achieved remarkable progress. Also the problem of isolating the bearers of the functions of the internal secretions and other physiologically active substances should receive benefit from this method. Medicine should, we hope, thereby be given new aids towards the curing of the diseases caused by disturbances of the internal secretions, and towards moderating the manifestations of old age.

The intellectual and the political leaders of peoples have not been able to prevent the sacrifice—as it seems to me, without possibility of ethical gain—of so many lives in our time, of millions of human beings in the flower of existence. Medicine, alone, in union with Science, can make amends for the errors of the governments, by protecting and saving innumerable human lives. To be sure, but a few individual human beings can be decisive in the moral and intellectual progress of the race. Yet each of its individual members is given precious possibilities. Be our own fate what it may, from each of us there can arise, whether as son or as grandson, another William Harvey.

THE EXCHANGE OF MATERIAL BETWEEN THE ERYTHROCYTE AND ITS SURROUNDINGS[1]

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MOST scientific work of a quantitative nature is concerned with the determination either of positions of equilibrium or of rates. In the case of the erythrocyte, most of the existing work falls into the first category. A large number of studies have been made on such things as the amount of oxygen that can be carried at a given oxygen tension, the volume assumed in a solution of given osmotic pressure, or the distribution of ions at a given pH value. But the rates at which these various equilibrium conditions are reached or approached by the exchange of substances between the erythrocyte and its surroundings, though in many ways of equal physiological interest, have as yet received much less attention. The present lecture will deal with this side of the question, with particular emphasis upon certain differences between the erythrocyte and other less highly specialized cells.

The greater part of our scanty knowledge of the rates at which substances enter and leave erythrocytes has been obtained by chemical analyses, made at intervals, of the liquid in which the cells are suspended. For example, Masing (1) obtained in this way data on the effects of temperature on the rate of penetration of the erythrocyte by glucose, and a few similar studies have been made by other workers. However, the considerable length of time required to separate the corpuscles from the plasma permits the use of the methods of analytical chemistry only in the case of very slowly penetrating substances. Physico-chemical methods involving measurements of freezing-points and of electrical conductivity have also been used in a number of cases, but without yielding any very complete or very exact data.

[1] Lecture delivered May 7, 1927.

There is, however, one substance regarding which information is now available, and fortunately it proves to be the most important of all of those with which the erythrocyte comes into relation, namely, oxygen. It has been recently found possible by Hartridge and Roughton (2) to measure with considerable accuracy the rate of intake of this substance by the erythrocyte. The method employed is both ingenious and simple. It depends upon the maintenance of a steady flow at a known rate, through a suitable vessel, of an oxygen-containing solution. At a given point another steady stream of reduced corpuscles is introduced and immediately mixed with the first stream. Since, in spite of the movements of the individual corpuscles, a stationary condition of the system as a whole is maintained, it is only necessary to measure the distance from the point of introduction of the corpuscles to the point where spectroscopic examination shows the hemoglobin to be saturated with oxygen to any desired degree. The rate of flow being known, this distance can then readily be converted into time.

The rate of entrance of oxygen into the erythrocyte turns out to be very rapid. With an oxygen tension of one-third of an atmosphere the hemoglobin in the interior of the corpuscle becomes half-saturated in approximately $\frac{1}{100}$ second. Rapid as this rate is, however, it is about ten times as slow as that found in the case of the hemoglobin from laked corpuscles. The most plausible, though perhaps not the only, interpretation of this difference is that the oxygen is measurably retarded in passing through the surface of the erythrocyte. The enclosure of the hemoglobin in formed structures, therefore, though highly advantageous in most respects, as pointed out by Barcroft (3) in a previous Harvey Lecture, is perhaps not without at least this theoretical disadvantage.

It is not possible to give figures of similar exactness for the rate of entrance of oxygen into other less specialized cells. It seems likely, however, that conditions for diffusion would be especially favorable in a structure having the dimensions and the shape of an erythrocyte. Not only does the small size give a high ratio of surface to volume, but the shape itself is a very advantageous one.

This question has been discussed by Hartridge (4), who points out that the greatest carrying capacity for the smallest amount of structural material would be furnished by a sphere. On the other hand, the most favorable conditions for diffusion would be present in an infinitely thin disc. The erythrocyte, in a sense, represents a compromise between these two forms, with the additional valuable feature of an increased thickness near the periphery where diffusion can occur from three directions. Ponder (5) has recently shown by a mathematical treatment that the shape of the erythrocyte is nearly, though not quite, the most effective possible from the point of view of diffusion. He has calculated that to secure as favorable conditions with spheres it would be necessary to divide an erythrocyte into nine spheres, each having one-ninth of the original volume.

In addition to substances like glucose, whose rate of entrance is slow enough to be followed by chemical methods, and to rapidly penetrating gases such as oxygen and carbon monoxide, whose compounds with hemoglobin have spectroscopic characteristics which permit the use of the method of Hartridge and Roughton, there are large numbers of other materials regarding which the available information of a quantitative sort is very imperfect. One of these is water, a substance whose importance is sufficiently indicated by the fact that it comprises over 60 per cent of the weight of the erythrocyte and an even higher percentage of that of the surrounding plasma. Studies of osmotic equilibria in which water is involved have been made in great numbers but the rates at which these equilibria are approached have been very generally neglected.

Fortunately there is available a very simple method of obtaining at least approximate information regarding the rate of entrance of water into the erythrocyte. This method depends on the swelling, followed by hemolysis, which occurs in pure water or in any sufficiently hypotonic solution. In many cases it may be shown by appropriate control experiments that the hemolysis produced in this way is so largely due to simple osmotic swelling that other factors may, as a first approximation, be neglected. In such cases—and it is only cases of this sort that will be dis-

cussed in this lecture—the point of hemolysis may be considered to represent the attainment of a certain degree of swelling, or, in other words, the entrance of a definite amount of water. Since different individual erythrocytes show somewhat different degrees of resistance to hemolysis, it is necessary to arrange an experiment in such a way that only a relatively homogeneous group of cells is dealt with.

The usual methods of following hemolysis, because of their slowness, are not well adapted to cases where the process is completed in a few seconds or sometimes even in a fraction of a second. It is not difficult, however, to devise methods by which such studies can be made. One type of apparatus that has been found useful for this purpose is a modification of that of Hartridge and Roughton. With it, it has been possible to deal with cases of hemolysis as rapid as that of sheep corpuscles in water and very dilute solutions where the time required is perhaps a third of a second or even less. When the times involved exceed one second, as is the case with human and with most other mammalian erythrocytes, an even simpler method gives good results. This method is as follows: A small quantity of corpuscles (usually a single drop of a fairly heavy suspension or sometimes of defibrinated blood) is suddenly mixed in a funnel with 25 cc. of the solution to be studied and allowed to flow rapidly into a vessel through which the image of the glowing filament of a carbon lamp is reflected by means of a mirror. By appropriate adjustments, the depth of the layer of the resulting dilute suspension of corpuscles through which the filament is viewed can be regulated as desired. It is then a simple matter with a stop-watch to determine the time required after the mixing in the funnel for a certain degree of hemolysis to appear. The nature of the method ensures that the time so determined is a measure of the rate of destruction of a small group of corpuscles of approximately equal resistance, whose disappearance just permits the filament to become visible.

The rate of entrance of water into the erythrocyte as determined in this way varies with circumstances. It is, of course, more rapid from strongly than from only slightly hypotonic solutions, and the experimental evidence favors the view that the rate

at any given instant is, other things being equal, proportional to the difference in osmotic pressure between the erythrocyte and the surrounding solution. If it be assumed that this relation is true, then the hypothetical rate of increase in volume can be expressed by the equation:

$$dV = kA(p - P)dt$$

where A is the surface area of the corpuscle, p its osmotic pressure, V its volume, P the osmotic pressure of the surrounding solution, and t the time. If the further assumptions be made that the ideal relation between osmotic pressure and volume holds with sufficient accuracy for the erythrocyte, and that because of its biconcave shape a considerable degree of swelling is possible without change in surface area and that A is therefore constant, the above equation becomes on integration:

$$k = \frac{p_0V_0}{P^2At} \ln \frac{p_0V_0 - PV_0}{p_0V_0 - PV} - \frac{V - V_0}{PAt}$$

where p_0 and V_0 are the initial osmotic pressure and volume, respectively, of the corpuscle. When the external medium is water and P is therefore zero, the resulting equation is:

$$k = \frac{V^2 - V_0^2}{2\ p_0V_0At}$$

The constant k in each of these equations, represents, as will be seen by inspection of the original differential equation, the amount of water that will enter in unit time with a unit difference in osmotic pressure, through unit area, and is therefore a satisfactory measure of the fundamental permeability of the corpuscle to water. The value of this constant depends in an interesting way on the character of the electrolytes present in the surrounding medium, but omitting the complications due to this factor, it may be said that its value for human erythrocytes is of the order of magnitude of 3.0, i.e., with a difference of osmotic pressure of one atmosphere three cubic micra per minute enter through each square micron of surface.

It is of interest to compare the rate of entrance of water into another type of cell with that into the erythrocyte. Figures for comparison are available for the egg of the sea-urchin *Arbacia*, which has been studied in this connection by Lillie (6) and more recently by McCutcheon and Lucké (7). According to the observations of Lillie the fertilized egg of *Arbacia* when placed in a mixture of 40 parts of sea water and 60 parts of tap water gains in the first minute 38 per cent of its initial volume, which corresponds to an average rate during this period of one-third of a cubic micron per minute, per square micron, per atmosphere. The rate of entrance at the first instant, when the full difference of osmotic pressure of 13 atmospheres is effective, is of course somewhat greater. On the assumption that the equation already given for the erythrocyte holds approximately for the first part of the swelling process when the change in the surface of the egg is relatively small, a value of k of the order of magnitude of 0.5 cubic micron per square micron, per minute, per atmosphere is obtained. For the unfertilized egg the corresponding figure is considerably less.

Expressed somewhat differently, if the rate of swelling corresponding to a difference in osmotic pressure between the cell and its surroundings of one atmosphere could be maintained constantly, the fertilized *Arbacia* egg would gain its own volume in twenty-five minutes. The erythrocyte under the same conditions would gain its own volume in fifteen seconds. A large part of this difference is of course due to the much greater surface: volume ratio in the case of the erythrocyte; but even when allowance is made for this factor by comparing the two velocity constants themselves, and when due account is taken of the lack of strict validity of the assumptions used in obtaining these constants, and also of possible inaccuracies in the measurements of the cells, it still seems likely that under comparable conditions the rate of penetration of water is several times greater in the case of the erythrocyte than in that of the egg of *Arbacia*.

The method of hemolysis can also be used to follow the penetration of dissolved substances known by control experiments to be inert except through the osmotic effects to which they give rise. Suppose, for example, that an erythrocyte be placed in a large

volume of an isotonic solution of glycerol. As the glycerol penetrates, its osmotic effect inside and outside of the corpuscle tends to approach a balance, leaving the dissolved substances in the interior unbalanced. Swelling therefore occurs, followed finally by hemolysis. Now, in general, if the internal osmolar concentration of salts in the corpuscle be represented by c and the external osmolar concentration of glycerol by C, then it is easy to see that when the cell in swelling has reached n times its original volume (which for simplicity may be taken as unity) the internal concentration of salts will have become c/n and the internal concentration of glycerol $C - c/n$. The total amount of glycerol that has entered will be $nC - c$. If, therefore, the value of n corresponding to hemolysis be determined by hematokrit readings or even more simply, though less accurately, by calculation from the strength of the solution just required to produce hemolysis, the amount of the diffusing substance that has entered up to the time of hemolysis may readily be estimated.

Specifically, for ox corpuscles we may take 1.5 as a possible value for n and 0.3 M as a possible value for c. When such corpuscles are placed in a large volume of, for example, a molar solution of glycerol, hemolysis should therefore occur when the internal concentration of this substance has reached 0.8 of the external concentration and when the *amount* which has entered has become equal (expressed in osmolar terms) to four times that of the substances originally contained in the cell. The extent of penetration required to produce hemolysis under these conditions is therefore very considerable.

Where the rate of entrance of the penetrating substance is slow compared with that of water, the mathematical calculations necessary to give the rate of entrance for unit difference between external and internal concentration are easy. Where the rate is of the same order as that of water, these calculations are much more difficult, and for simplicity there will therefore be given merely the times required to produce hemolysis of ox corpuscles in solutions of different strengths of three typical non-electrolytes: ethyl alcohol, urea and glycerol. In the case of the molar solutions, hemolysis in each case indicates, as has just been stated, the

penetration of an amount of the substance in question equivalent osmotically to approximately four times the amount of the dissolved substances originally present in the erythrocyte. For the other concentrations the corresponding figures may easily be calculated.

It is of interest to compare the behavior of these substances in entering the erythrocyte with that observed in the case of other cells. So far as ethyl alcohol and glycerol are concerned, erythrocytes are very similar to various plant and animal cells studied by Overton (8) and others. Ethyl alcohol in every case enters

TABLE 1

Time required for hemolysis of ox corpuscles in solutions of ethyl alcohol, urea and glycerol

CONCENTRATION	TIME		
	Alcohol	Urea	Glycerol
	seconds	*seconds*	*seconds*
M/1	1.7	5.7	8400
M/2	1.5	3.0	4800
M/4	1.2	1.9	2040
M/8	1.0	1.3	2.8
M/16		1.3	1.4
M/32		1.2	1.3
Water		1.0	1.1

with extreme rapidity and glycerol much more slowly, though at a measurable rate. Urea, on the other hand, shows a very different behavior when the erythrocyte is compared with other cells. To mention only one example, Collander (9) has recently compared the relative ability of a number of substances to enter certain plant cells, and his figures show that the penetration of urea into these cells extends over a period of a good many hours, the rate being, as a matter of fact, considerably slower than that of glycerol. Overton and others had previously reported similar findings for other cells. The erythrocyte, on the other hand, offers almost no opposition to the penetration of this substance. It appears therefore that it is dangerous to draw conclusions as

to the permeability of cells in general from studies made on one particular type of cell such as the erythrocyte. In some cases, as with alcohol and glycerol, such conclusions might be fairly correct, but in others, as with urea, they would be utterly misleading.

We may go one step farther. It is not even permissible to draw conclusions about the behavior of erythrocytes in general from that of a single kind of erythrocyte. For example, glycerol enters the corpuscles of the sheep and the ox very slowly. A half-molar or molar solution will produce hemolysis only after from one to several hours. The same result is obtained with human corpuscles in a few minutes, other animals on which studies have been made, including the dog, cat, rat, mouse, guinea pig and bat, falling between the two extremes. This difference is not due to possible differences in the resistance to swelling of the various corpuscles, since, as a matter of fact, the human corpuscles which hemolyze most quickly in glycerol are among the most resistant to simple hypotonic swelling, while with those of the ox and sheep the reverse is true. In this connection, it is interesting to note that the human erythrocytes which are most easily penetrated by glycerol, appear also to be unique in the relatively low resistance they offer to the entrance of dextrose (10, 11). This fact is possibly significant in view of the known chemical relationships of the two substances.

Among other chemical compounds which have been studied in the manner described, the ammonium salts are of considerable interest, not so much on account of their own intrinsic importance as because their behavior both illustrates in a very clear manner certain fundamental peculiarities of cell permeability in general and, in particular, brings out an important difference between the erythrocyte and all other cells which have so far been investigated. To make clear the probable physiological significance of this difference, a short digression will be necessary, after which the behavior of the salts in question may be considered in some detail.

The primary function of the erythrocyte is, of course, the transport of oxygen. Like a boat, it takes up a cargo of this substance in the lungs, carries it to the tissues and discharges it and then returns to the starting point for more. Now a cargo-carrying

boat is usually so managed that it does not go back empty for a new cargo but utilizes its return voyage to carry some different sort of material. Similarly, the erythrocyte does not return to the lungs merely "in ballast," but performs on this part of the trip another function second in importance only to the transport of oxygen, namely, assistance in the preservation of a constant blood reaction during the transport from the tissues and elimination in the lungs of carbon dioxide. Thanks to the erythrocyte the blood may alternately be flooded with and lose large quantities of this substance with only infinitesimal changes in reaction.

The manner in which the erythrocyte participates in the transport of carbon dioxide is so well known that it need only be referred to briefly by way of explaining certain points which will be discussed in more detail later. The conditions existing in the blood may be summarized as follows: In the plasma there are found the ions of various neutral salts, chiefly NaCl, which may for convenience be designated as $B^{\cdot}$ and Cl'. In addition there are present the ions $B^{\cdot}$ and HCO_3' from the plasma bicarbonates, $H^{\cdot}$ and OH', which always occur in aqueous solutions, and undissociated carbonic acid, represented for simplicity entirely as CO_2. The pH of the plasma may be considered for practical purposes to be determined by the ratio of CO_2:HCO_3' and will, other things being equal, rise and fall with fluctuations in the carbon dioxide tension in a characteristic manner.

Within the erythrocyte we also have the ions $B^{\cdot}$, Cl', HCO_3', $H^{\cdot}$ and OH', together with undissociated CO_2. In addition there is present hemoglobin, which in blood coming from the lungs may be assumed for simplicity to be completely oxygenated. Part of this oxyhemoglobin is in the form of an undissociated acid, which may be represented as $HHbO_2$, and part is in the form of a dissociated salt whose ions are $B^{\cdot}$ and HbO_2'. As in the plasma, any given pH is associated with a definite ratio of CO_2:HCO_3' and in addition with another equally definite one of $HHbO_2$:HbO_2'. When carbon dioxide passes into the blood from the tissues it tends, by changing the ratio of CO_2:HCO_3', to increase the acidity of the plasma. Some of it, however, enters the erythrocytes and by taking up base formerly combined with the hemoglobin yields

HCO_3' ions paired with $B^{\cdot}$ ions and at the same time increases the amount of $HHbO_2$. This process is greatly facilitated by the fact that at the time when carbon dioxide is entering the blood oxygen is leaving it, and reduced hemoglobin, being a weaker acid than oxyhemoglobin, parts with its base more readily.

The mechanism just described is an extremely efficient one, but is capable of being, and has been, further improved in the mammalian body. In spite of the combination in the corpuscle of much carbon dioxide with base formerly held by hemoglobin, the tension of plasma CO_2 rises slightly in the tissue capillaries. The effects of this rise might readily be counteracted by a simultaneous increase of HCO_3' if the extra bicarbonate already found in the corpuscle could in some way be transferred to the plasma. However, both osmotic and electrical conductivity studies indicate that the erythrocyte is impermeable to salts as such, and, indeed, without this impermeability certain of the great advantages of the erythrocyte as a carrier of oxygen emphasized in a previous Harvey Lecture by Barcroft (3) would be lost.

Under these circumstances, the body has found an arrangement which combines all of the advantages and none of the disadvantages of a cell permeable to ions in general. This is a condition of permeability to anions only. Such an arrangement prevents the passage of salts as such, but at the same time permits HCO_3', when its amount in the erythrocyte increases, to be exchanged for Cl' from the plasma. This efficient device is the one which appears to have been adopted by at least the majority of the vertebrates. The well-known "chloride shift" which occurs when carbon dioxide is added to whole blood is a visible evidence of its presence. To the structural and chemical peculiarities which differentiate the erythrocyte from other types of cells must be added this equally striking type of permeability to ions. It is true that the distinction between the erythrocyte and other cells may be one of degree only. The recent work of Smith (12, 13) indicates that in various other cells there may be under certain conditions at least an apparent exchange of anions between the cell and its surroundings. However, no other cell, so far as is known at present, shows anything like the same development of this "differential permeability" as does the erythrocyte.

The unique properties of the erythrocyte may now be illustrated in a striking way by comparing its behavior with that of certain other cells in solutions of ammonium salts. Consider first an ordinary cell in an isotonic solution of, for example, NH_4Cl. The essential characteristics of such a solution are represented in figure 1. In addition to the ions $NH^{\cdot}$ and Cl' there have been formed in the solution by hydrolysis, in the manner indicated in the diagram, dissociated hydrochloric acid, which gives to it an acid reaction, and undissociated NH_3. (For the sake of simplicity no attempt is made to distinguish between NH_3 and NH_4OH.) Of the various ions and molecules present, ordinary cells show a free permeability only to NH_3 and H_2O. It would be expected

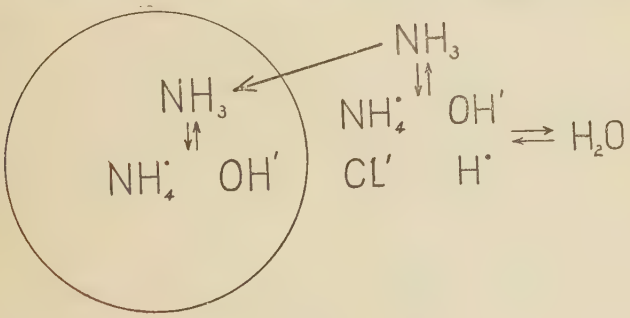

Fig. 1.

therefore, that even though the external solution be acid in reaction, it could, by means of the penetrating ammonia, produce an increased alkalinity or a decreased acidity within the cell. This expectation is realized in the case of a number of types of cells, both plant and animal (14). Since the amount of ammonia which must penetrate to establish equilibrium with the external solution is extremely small, no significant osmotic effects would be anticipated, and ordinary cells in isotonic solution of NH_4Cl, as a matter of fact, show volume changes which are negligible. In some unpublished experiments made with the assistance of Dr. E. M. Landis it was found that in the case of *Arbacia* eggs, whose volume changes can easily be measured, the effects of solutions

of NaCl, KCl and NH_4Cl were (except for the greater toxicity of pure NaCl) almost identical.

The behavior of the mammalian erythrocyte is entirely different. In isotonic solutions of NaCl and KCl it retains its original volume almost unchanged and remains intact under favorable conditions for days. In a similar solution of NH_4Cl, on the other hand, swelling begins immediately and leads within ten or fifteen minutes to hemolysis. That the hemolysis is essentially an osmotic one is shown by the readiness with which it can be prevented by adding suitable amounts of a non-penetrating substance such as NaCl, KCl or sucrose to the solution. It is also known from direct chemical analyses and freezing point determinations that

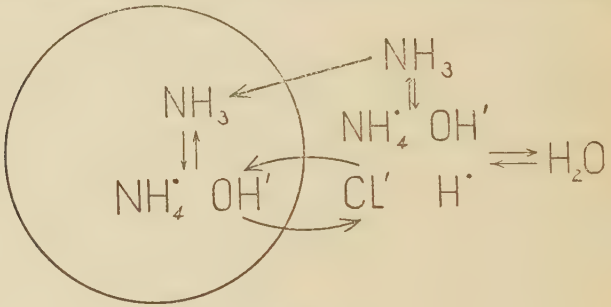

Fig. 2.

NH_4Cl enters the corpuscles. It is frequently supposed that the entrance of an ammonium salt occurs in the form of its ions, but if this were the case then the electrical resistance of a suspension of erythrocytes in a solution of NH_4Cl should be much less than in a similar solution of the non-penetrating salts, NaCl or KCl, and this appears not to be the case (15).

The probable reason for the unique behavior of the ammonium salt becomes apparent when the peculiar type of permeability of the erythrocyte is taken into account. In figure 2 are represented the possibilities for an exchange between a solution of NH_4Cl and an erythrocyte. Everything is the same as in figure 1 with one important exception. This is the permeability of the erythrocyte to anions. In any cell in a solution of NH_4Cl, there is, as has

already been indicated, a tendency for the internal concentration of OH′ to increase through the penetration of NH_3. But in the erythrocyte under these conditions there is not only the possibility but the necessity for an exchange (16), either directly or indirectly of some of the internal OH′ ions for external Cl′ ions, since the condition of equilibrium demands that

$$[OH]_{internal} \times [Cl]_{external} = [OH]_{external} \times [Cl]_{internal}$$

The passage of OH outward, however, disturbs the equilibrium in the cell between dissociated and undissociated NH_3 and thereby leads to the entrance of more of the latter. This process would (if hemolysis did not in the meantime intervene) continue until the NH_4Cl had distributed itself so as to produce a concentration within the corpuscle approximately equal to that without. The equilibrium approached in this indirect manner is evidently the same as if the corpuscle were permeable to NH_4Cl as such, since it is a well-known principle in thermodynamics that the position of an equilibrium is not affected by the manner in which it is approached.

The unique hemolytic effect of ammonium salts may therefore be explained by the coincidence of two rather unusual things: a salt which by hydrolysis furnishes a readily penetrating substance, NH_3, and a cell which is permeable to anions. If either the salt or the type of cell be changed the characteristic swelling on which hemolysis depends disappears. In this connection, it is of interest to note that the hemolytic effect of NH_4Cl, which is found in all the mammalian erythrocytes so far studied and also in those of the fowl, the turtle, the alligator and the frog, is according to some recent observations of Miss Morrison almost absent in the erythrocytes of at least certain fishes. Does this mean that the erythrocytes of this most primitive group of vertebrates lack or show to a lesser degree the peculiarity of ready permeability to anions found in the higher groups? Experiments are under way which will, it is hoped, furnish an answer to this question.

Lack of time forbids a detailed discussion of the effect of various factors on the rate of penetration of NH_4Cl and other similar

salts. It may merely be mentioned that changes in the pH of the solution affect the process in a most decided manner. For example, when the pH of the solution is reduced to 4.0 or less, ammonium chloride behaves exactly like sodium chloride at the same pH. The most plausible explanation of this fact is that in the practical absence of free NH_3 the process as pictured in figure 2 cannot occur. It is true that experiments of the type mentioned are complicated by the fact that to remove all but negligible amounts of free ammonia a degree of acidity is necessary which is in itself hemolytic. However, the important fact remains that under these conditions hemolysis occurs no more rapidly in solutions of NH_4Cl than in those of NaCl of the same concentration and pH. In more alkaline solutions, in addition to the effects described, there is an immediate shrinkage in the volume of the erythrocyte, such as is known generally to occur under these conditions. This volume change produces certain complications, but when proper allowance is made for them the results obtained are entirely consistent with the theory.

The only ammonium salt considered so far is one which by hydrolysis produces a strong and probably completely dissociated acid, HCl. It is known from the studies of many previous workers that such an acid shows only feeble powers of penetrating living cells. It is of interest to compare with salts of this type others which give rise to weak, largely undissociated acids whose penetrating powers are much greater. For this purpose the ammonium salts of formic, acetic, propionic, butyric and valeric acids have been studied. It is known that the ability of these acids to penetrate living cells, when the effects of injury are as far as possible ruled out, is in the order given, i.e., in that of increasing molecular weight, formic acid penetrating least and valeric acid most readily (17). A feature which aside from their general chemical relationship makes these acids of especial value is the fact that the last four possess almost exactly the same strength as acids. For this reason their ammonium salts are hydrolyzed to an equal degree and at any given pH each solution contains approximately the same amounts of undissociated ammonia and of undissociated acid as the others.

Figure 3 represents a cell in a solution of the salt of an acid of this type, for example, ammonium butyrate. The chief difference between this case and that where ammonium chloride is concerned is that the acid resulting from the hydrolysis of ammonium butyrate is largely undissociated and is able to penetrate living cells with ease. The result is that through the independent entrance of NH_3 and butyric acid, in the manner shown in the diagram, the salt rapidly accumulates in the cell, the equilibrium approached again being the same as if the cell were permeable to the salt as such or to both of its ions. Furthermore, penetration by salts of this type should theoretically not be confined to erythrocytes but should occur in all cells, since no cell is known which

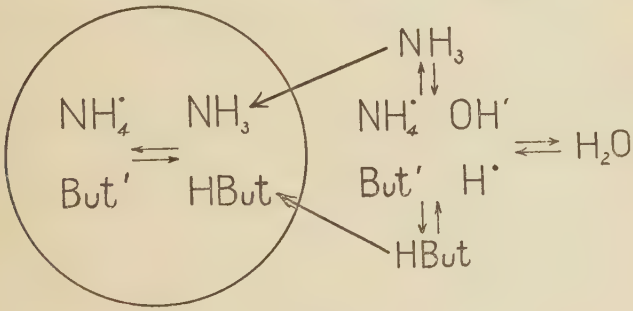

Fig. 3.

is not readily penetrated by NH_3 and by butyric acid. As a matter of fact, Miss Stewart has recently found that the ammonium salts of the fatty acids, unlike the chloride and nitrate, readily penetrate the eggs of *Arbacia* and Dr. R. Bouillenne during the past winter has observed the same behavior with a number of plant cells.

The rate of penetration in every case—erythrocyte, *Arbacia* egg and plant cell—is always in the order: formate < acetate < propionate < butyrate < valerianate. The following figures for the time of osmotic hemolysis of the erythrocytes of the ox are typical (table 2). It may be noted that the degree of hydrolysis and therefore the concentrations of the penetrating substances are almost equal in the case of the last four acids.

It is of interest in the case of salts of this type to study the effect on the rate of penetration of changing the pH of the solution. It would be expected that the rate would be most rapid when NH_3 and acid molecules enter the cell in equal numbers, since in this case they would exactly neutralize each other within the cell and

TABLE 2

Time required for hemolysis of ox corpuscles in M/4 solutions of ammonium salts of fatty acids

SALT	TIME	
	At pH 7.0	At pH 6.5
	seconds	*seconds*
Ammonium formate	239	159
Ammonium acetate	88	39.8
Ammonium propionate	27.6	22.3
Ammonium butyrate	20.2	20.4
Ammonium valerianate	15.1	19.6

TABLE 3

Effect of pH on time required for hemolysis of ox corpuscles in M/4 ammonium acetate

pH	TIME	pH	TIME
	seconds		*seconds*
4.8	1260	7.4	258
5.0	600	7.8	600
5.2	260	8.3	1260
6.0	53	8.6	1740
6.5	59	9.0	3000
7.0	109		

so preserve a steep diffusion gradient across the cell boundary. An equal rate of penetration of the two molecules would of course not necessarily mean an equal concentration of the two in the external solution, but at any rate it could be predicted that, given the optimum external ratio, as determined by a certain pH, any change in the reaction of the solution in either direction should

give a slower rate. This proves to be the case, as is shown by the figures for M/4, i.e., somewhat hypertonic, ammonium acetate (table 3).

Essentially similar results have been obtained with the other four fatty acids. The retardation is, as would be expected, always greater on the alkaline than on the acid side of the optimum pH, since, as is well known, erythrocytes tend to shrink in alkaline and swell in acid solutions and these volume changes modify the course of those produced by the entrance of the salt. It is perhaps partly for this reason that the optimum pH for hemolysis by ammonium acetate lies considerably below that where the two penetrating molecules are equal in concentration. Another factor is probably the less ready penetration of the cell by acetic acid than by ammonia. In the case of the more readily penetrating butyric and valeric acids the optimum pH lies higher as would be expected.

The various facts that have been mentioned in this lecture relate only to a few of the many chemical compounds with which the erythrocyte normally comes in contact or whose behavior might for other reasons be of physiological interest. A large amount of work will be necessary before really exact quantitative data on the rates of exchange of more than a small number of substances are available. But even in the present state of our knowledge, which is at best only semi-quantitative and which applies only to a very few substances, it is possible to see that the rates at which materials enter and leave the erythrocyte are just as important and characteristic properties of the latter as are its better known structural and chemical peculiarities. If these rates have received less attention in the past, it is not because they are unimportant but because their study by exact methods is difficult, and because other matters have for various reasons appeared more pressing. But no physiologist can doubt the ultimate importance of studies of this sort, and it is to be hoped that in the near future much information on rates may be added to our already fairly satisfactory knowledge of the various equilibria in which the erythrocyte is involved.

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